THE NEURONAL SUBSTRATE OF HYPOTHALAMICALLY INDUCED BEHAVIOR IN THE RAT

An anatomical and behavioural study

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DR. M.R. KRUK (RIJKSUNIVERSITEIT LEIDEN)

Only for you, children of doctrine and learning, have we written this work. Examine this book, ponder the meaning we have dispersed in various places and gathered again, what we have concealed in one place we have disclosed in another, that it may be understood by your wisdom

H C A. von Nettesheim, De occulta philosophia 3, 65
U Eco, Foucault's pendulum

Aan mijn familie

Aan mijn vrienden

CONTENTS

PART 1 INTRODUCTION

1.1 INTRODUCTION TO THE MORPHOLOGY AND FUNCTION OF THE HYPOTHALAMUS

The study of brain mechanisms regulating behaviour has become more and more of a multidisciplinary character, involving cell biology, neuroanatomy, ethopharmacology, physiology, endocrinology and molecular biology. Within the progress of knowledge of these mechanisms, it has become clear, that their morphological substrate is not formed by discrete centres, located within discrete cyto-architectonical areas of the brain, but rather consists of intricate complexes of neurons extending through many parts of the brain that are interconnected by diffusely arranged sets of fibres [60,83].

Within the circuitry involved in the regulation of behaviour, the hypothalamus plays an important role [83]. The fact that different more or less complete types of behaviour as well as several different autonomic responses can be elicited by stimulation of different parts of the hypothalamus, indicates that the hypothalamus must be regarded as an important part of the morphological substrate underlying these mechanisms [35,56,83]. Through an abundant set of afferent and efferent connections with limbic and other parts of the brain and with the autonomic nervous system and the endocrine system, the hypothalamus is capable of integrating sensory input and eliciting co-ordinated behavioural, autonomic and neuroendocrine responses (fig. 1) [56,57,83].

In this introduction, a brief overview will be presented of the structure of the rat hypothalamus. Some functions of the hypothalamus will be considered, as well as the way in which different parts of the hypothalamus are involved in different aspects of these functions. Some brain circuitries involved in the regulation of different behavioural responses will be discussed.

1.1.1 Historical background and general subdivisions

The designation hypothalamus was first used by His (1885) [30] and later by Kölliker (1896) [37] and Edinger (1896) [20] to denote a part of the brain positioned ventral to the thalamus, which merges rostrally with the preoptic region and caudally with the ventral tegmentum and the mesencephalic central gray without clear cytoarchitectonic boundaries [57]. Gurdijan (1927) [27], Krieg (1932) [38] and Le Gros Clark (1938) [16] have contributed to the cytoarchitectonic subdivision of the hypothalamus. Based on the work of Crosby and Woodburne (1940) [18] and Le Gros Clark (1938) [16], the hypothalamus is nowadays generally subdivided into four rostrocaudal levels and three mediolateral zones [13,57,83]. From rostral to caudal, a preoptic, an anterior or supraoptic, a tuberal and a mammillary level have been distinguished. The preoptic level contains *inter alia* the medial and lateral preoptic areas. The anterior, or supraoptic level comprises amongst others the anterior and supraoptic hypothalamic nuclei. The tuberal level is located at the level of the ventromedial and dorsomedial hypothalamic nuclei. The posterior level includes the mammillary

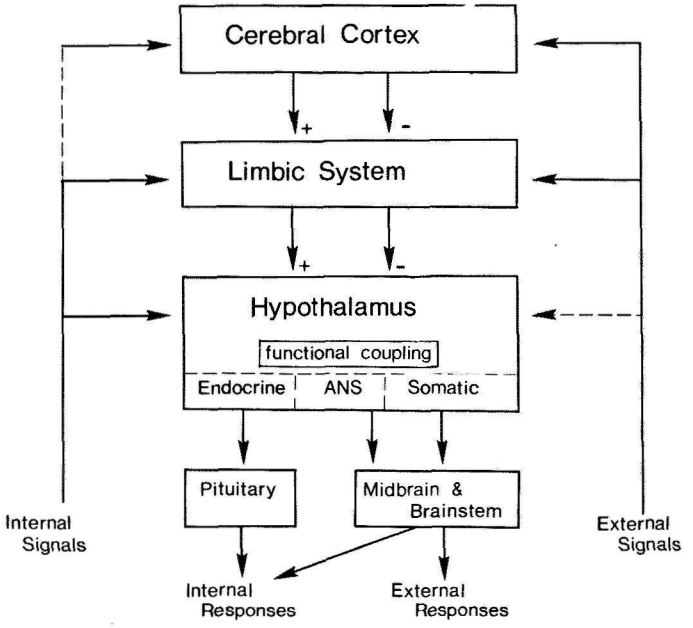


Figure 1: *Schematic model of the role of the hypothalamus in the circuitry of internal and external responses to internal and external signals. Redrawn from Mogenson and Huang (1973)* [56].

nuclei, supramammillary nuclei and posterior hypothalamic area. From medial to lateral, a periventricular, a medial and a lateral zone have been distinghuished. A dorsal region is sometimes added as an area overlying the other zones [13]. The mediolateral zones are best noticable at the tuberal level of the hypothalamus [83]. The periventricular zone contains the periventricular, hypothalamic paraventricular, suprachiasmatic, and arcuate nucleus. The medial zone includes the ventromedial (VMH) and dorsomedial (DMH) hypothalamic nuclei. The lateral zone is situated laterally from the fornix and consists of scattered neurons among the fibres of the medial forebrain bundle.

Recently, Geeraedts et al. [22] have introduced a new cytoarchitectonic subdivision of the hypothalamus. They added an intermediate hypothalamic zone, containing the

intermediate hypothalamic area (IHA), the perifornical area (PFX), the lateral supramammillary nucleus (SUL) and the tuberomammillary area (TUM) [22]. The lateral hypothalamic zone, which for a long time had been regarded as a continuum of diffusely arranged neurons [57,83], was subdivided by these authors into a number of nuclei and areas [21,22].

In figure 2, a diagrammatic horizontal section is presented through the hypothalamus, largely based on the work of Geeraedts et al. [21,22]. The intermediate zone is separated from the medial and lateral zones by hatched lines.

1.1.2 Morphology of hypothalamic neurons.

The thorough Golgi studies of the hypothalamus performed by Millhouse (1979) have indicated, that hypothalamic neurons in the lateral and medial zones have many features in common: neurons from the lateral hypothalamic zone are usually small with long, poorly ramifying dendrites, crossing the borders of the cytoarchitectonically defined zones and levels of the hypothalamus [53]. Within the set of neurons from nuclei in the medial zone a larger number of medium sized neurons are found [53]. The dendrites of neurons in lateral and medial zones are interwoven and thus form a reticulum spanning the hypothalamus in the transversal plane. In between these dendrites, numerous rostrocaudally oriented fibres are found, forming the medial forebrain bundle in the lateral zone [59,90]. Although in Nissl preparations the hypothalamic ventromedial nucleus appears to be separated from the surrounding hypothalamic areas by a cell-sparse zone, Golgi studies have shown that the dendrites from VMH neurons radiate in all directions and far beyond the cytoarchitectural boundaries of that nucleus [52].

The dendrites of the neurons situated in the periventricular zone are in general not as elongated as those of the neurons in other hypothalamic areas. Especially dendrites from the suprachiasmatic nucleus do generally not extend beyond the cytoarchitectonic boundaries of this nucleus [53]. Of the arcuate nucleus only some dendrites cross the borders of the nucleus. Within the paraventricular hypothalamic nucleus (PVH) dendrites of the neuroendocrine magnocellular neurons are mainly confined to the nucleus itself [6,65]. Dendrites from the parvocellular PVH neurons are more elongated and sometimes cross the borders of the PVH [7,79,89]. Second order dendrites from PVH neurons form a thin halo around the nucleus, within the cell-sparse zone ventral and lateral to the nucleus [65,89].
Taken together, the hypothalamus gives the impression of a reticulum of neurons with long dendrites spanning the hypothalamus in the transversal plane. Many neurons in the periventricular zone differ morphologically from neurons in the other zones, which may well be related to the specific function of this zone in the regulation of the pituitary.

1.1.3 Connections of the hypothalamus

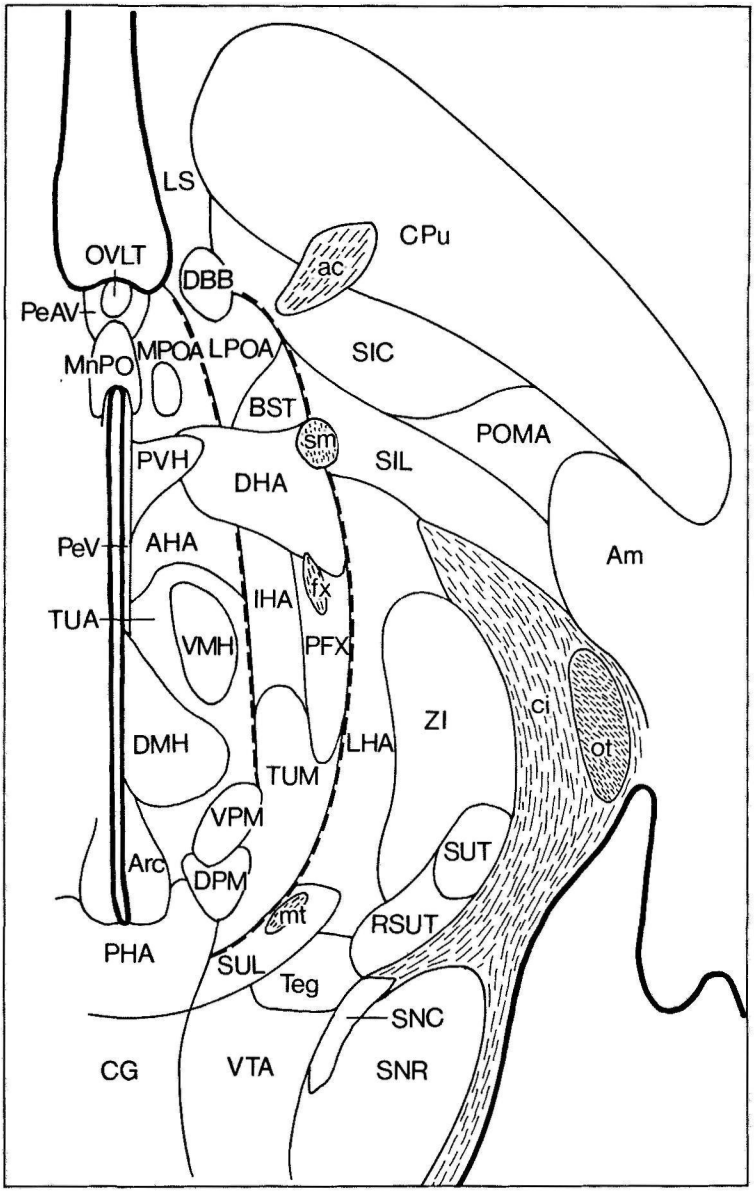
The hypothalamus is connected with a large number of brain areas that are known to regulate different aspects of behavioural, autonomic and endocrine responses to internal and external stimuli [83]. Projections entering or leaving the hypothalamus are situated mainly in four major fibre streams: the medial forebrain bundle, the periventricular fibre

system, the fornix and the mammillothalamic tract [59 83 90]. Both the medial forebrain bundle and the periventricular fibre system appear to be important input and output pathways of the hypothalamus for its role in the regulation of behaviour [83]. The medial forebrain bundle and the periventricular fibre system consist of diffusely arranged fibres and both differ in that respect from the fornix and the mammillothalamic tract [59 83]. The medial forebrain bundle is positioned in the lateral hypothalamic zone and consists of fibres originating in and projecting to a large number of brain areas [59 90 91]. The periventricular fibre system, which, as its name indicates, is situated in the periventricular zone surrounding the third ventricle, consists of diffusely arranged sets of ascending and descending fibres [83].

The hypothalamus receives afferent projections from a large number of brain areas, among which several nuclei of the amygdala, the septal nuclei, the bed nucleus of the stria terminalis, the central gray, autonomic nuclei in the brain stem and the raphe nuclei [83]. The extent to which different parts of the hypothalamus receive afferent projections from these brain areas may differ [12 90 94]. Next to afferent projections from these brain areas, the hypothalamus receives afferent information from the circumventricular organs [3 54 96].

The hypothalamus projects to many of the brain areas, from which it receives afferent information as well [83]. Some of these areas, like the central gray [10 91 93], parabrachial nuclei [55 91], pontine nuclei [1], septal nuclei [91], raphe magnus [32 92] and dorsal vagal complex [91], receive afferent projections from only specific parts of the hypothalamus. Parts of the hypothalamus project to the circumventricular organs as well [45]. Within the hypothalamus itself, many intrahypothalamic connections, e.g. between the lateral hypothalamic area, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus and dorsomedial hypothalamic nucleus, have been observed [46 49 69 84 85]. The largest projection to the neuroendocrine system arises from the periventricular zone but neurons in other hypothala-

Figure 2: Schematic horizontal presentation of the major subdivisions of the hypothalamus, largely based on Geeraedts et al. [21 22]. The medial, intermediate and lateral zones of the hypothalamus are separated by dashed lines. Abbreviations: ac: anterior commissure; AHA: anterior hypothalamic area; Am: amygdala; Arc: arcuate nucleus; BST: bed nucleus of the stria terminalis; ci: capsula interna; CG: mesencephalic central gray; CPu: caudate putamen; DBB: diagonal band of Broca; DHA: dorsal hypothalamic area; DPM: dorsal premammillary nucleus; fx: fornix; IHA: intermediate hypothalamic area; LHA: lateral hypothalamic area; LPOA: lateral preoptic area; LS: lateral septal nucleus; MnPO: median preoptic area; MPOA: medial preoptic area; mt: mammillothalamic tract; ot: optic tract; OVLT: organum vasculosum of the lamina terminalis; PeAV: anteroventral periventricular nucleus; PeV: periventricular nucleus; PFX: perifornical nucleus; PHA: posterior hypothalamic area; POMA: magnocellular preoptic nucleus; PVH: paraventricular hypothalamic nucleus; RSUT: retrosubthalamic nucleus; SIC. substantia innominata, pars subcommissuralis; SIL: substantia innominata, pars sublenticularis, sm· stria medullaris, SNC: substantia nigra, pars compacta; SNR: substantia nigra, pars reticulata; SUL: lateral supramammillary nucleus; SUT. subthalamic nucleus; Teg: mesencephalic tegmentum; TUA area of the tuber cinereum; TUM: medial tuberal nucleus; VMH: ventromedial hypothalamic nucleus; VPM. ventral premammillary nucleus; VTA: ventral tegmental area, ZI: zona incerta.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mic areas may project to the pituitary as well, e.g. the lateral hypothalamic area, and the lateral and medial preoptic areas [5]. Next to projections towards the median eminence and the pituitary, the periventricular zone sends projections to the autonomic centres in the brain stem [7,34,62,79,82,83].

Taken together, it can be concluded that within the hypothalamus a preferential organization of afferent and efferent connections is present. This preference in connections between specific parts of the hypothalamus and specific nuclei elsewhere in the brain is probably related to the specific function of each particular part of the hypothalamus.

1.1.4 Functions of the hypothalamus

The hypothalamus is considered to play an important role in the integration of information from peripheral and central sense organs and the execution of an integrated autonomic, endocrine and behavioural response to these stimuli in order to ensure the survival of both the individual and the species [83]. Different functions have been suggested for the different longitudinal zones [83]. The periventricular zone is primarily involved in the regulation of the neuroendocrine system by its extensive efferent projections to the pituitary and the median eminence. However, the periventricular zone, and especially the hypothalamic paraventricular nucleus, has also extensive connections with brainstem nuclei and parts of the spinal cord that are involved in autonomic control of a variety of body functions, such as cardiovascular, intestinal and respiratory regulation [6,47,49,67,68,79,80,82,83]. The medial zone has been suggested to be involved in the integration of information from the limbic system, whereas the lateral zone is suggested to be involved in behavioural responses (see below) [83]. However, on the basis of its connections, the lateral zone may be very well capable of integrating limbic information, whereas stimulation of the medial zone has been reported to elicit different types of behaviour as well (see below). A distinct differentiation between the functions of the different hypothalamic zones, as suggested by Swanson [83], is therefore not likely.

1.1.4.1 Hypothalamus and cardiovascular regulation

Electrical stimulation of different parts of the hypothalamus has been reported to elicit cardiovascular responses. The type of response is dependent on the site of stimulation: pressor responses are evoked by electrical stimulation of the ventral parts of the hypothalamus, while depressor responses occur in response to stimulation of the dorsal parts [98]. The injection of various substances has indicated that hypothalamic neuronal cell bodies are involved in at least part of the response [2,23]. However, local injections with excitatory amino acids have shown that the spatial organization of pressor and depressor responses is more mediolaterally organized instead of dorsoventrally [2]. Depressor responses can be elicited by injection into the lateral part of the hypothalamus and pressor responses can be elicited by injection into the medial and intermediate hypothalamic areas [2,23]. In the posterior hypothalamus, pressor responses occur after injection of GABA-antagonists and excitatory amino acids [9,61,95]. The role of the periventricular hypothalamic zone, in particular the paraventricular hypothalamic nucleus, in cardiovascular regulation is

less clear. Electrical stimulation of the parvocellular division of the hypothalamic paraventricular nucleus and infusion of this area with the glutamate receptor agonist kainic acid elevates both heart rate and blood pressure [63,64] while infusion of L- glutamate or low intensity electrical stimulation decreases blood pressure [36,97] Discrepancies between these results have been attributed to experimental circumstances, like choice of analgetics, or to stimulation of different functional units of the PVH [19,28,33,64].

1.1.4.2 The hypothalamus and behaviour

In 1943, Hess and Brugger emitted a large report on the elicitation of defensive behaviour by electrical stimulation of parts of the hypothalamus of cats [29] Since the behavioural response was largely complete, it was regarded to be of a motivational rather than of a motoric origin [29] In the years thereafter, these experiments have been repeated and extended by other investigators A number of other types of behaviour have been elicited by stimulation of parts of the hypothalamus, such as feeding, drinking, gnawing, food hoarding, and sexual behaviour Since different types of behaviour were elicited at different sites of the hypothalamus, the general view arose that within the hypothalamus, spatially organized centres exist for the control of specific motivational states, resulting in the stimulation of different behavioural responses

The spatial organization of functionally different sites within the hypothalamus was questioned by Valenstein and collegues (1969, 1970) [86,87] Their objection against the view of the spatial organization of hypothalamically elicited behaviour was based on three findings 1) hypothalamically elicited behaviour differs significantly from types of behaviour evoked by motivational states, such as hunger or thirst, 2) from the majority of electrode placements different types of behaviour (feeding, drinking and gnawing) could be elicited, 3) the distribution of electrode placements where one behaviour could be elicited, did not differ from the distribution of electrode placements where another behaviour was elicited [17,86,87,88]. Valenstein therefore concluded, that the behaviour produced by electrical brain stimulation is "in part the concomitant of the responses that tend to be dominant as a result of the interaction of such factors as environmental conditions, the presence of compelling stimuli and a particular aroused animal" (prepotency hypothesis) [86].

The conclusion of Valenstein was severely questioned by other investigators in the field, like Roberts [66], Bergquist [11] and Jurgens [35]. They based their objections on the specific properties of electrical stimulation as being non-selective, too gross to activate but a single behavioural circuit, or being only able to stimulate fragments of the motivational mechanism [11,66], or on the lack of an adequate environmental situation necessary to elicit the proper response [35]. The issue of non- selectivity of electrical stimulation was strengthened by the results of Grossman [24,25,26], who found that chemical stimulation of the same hypothalamic area with different neuroactive substances could evoke different types of behaviour Another explanation for the occurrence of different types of behaviour at the same electrode placement site was based on the findings of MacDonnell and Flynn [50,51], who observed activation of specific sensory fields on cat's muzzle during hypothalamic electrical stimulation The occurrence of such sensory field activation was also found in the rat during stimulation of hypothalamic areas, where feeding, drinking, gnawing and attack behaviour were elicited [74]. It was therefore suggested, that all four behavioural responses may be the result of a general activation of these sensory fields, while the

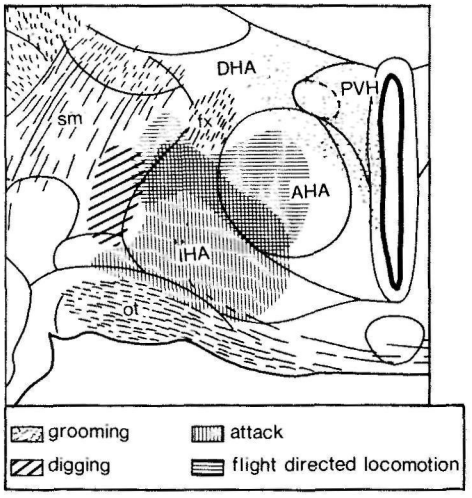

Figure 3: *Presentation of the spatial organization within the hypothalamus of electrical stimulation-induced digging, attack, flight- oriented locomotion and grooming. The behaviourally defined areas were redrawn from the discriminant analysis studies of Lammers et al.* [42,43,44] *onto the atlas of Geeraedts et al.* [21,22]. *Abbreviations: AHA: anterior hypothalamic area; DHA: dorsal hypothalamic area; fx: fornix; IHA: intermediate hypothalamic area; ot: optic tract; PVH: hypothalamic paraventricular area; sm: stria medullaris.*

choice of the response was dependent on other factors [74]. In a review on hypothalamically elicited behaviour, Jürgens [35] stated that the more related two behavioural functions are, the greater the overlap in their hypothalamic representation is.

In 1987 and 1988, Lammers et al. [42,43,44] reinvestigated the spatial organization of hypothalamically induced behaviour, using electrical stimulation in an enriched environment to enable the experimental animal to do whatever it may prefer. Furthermore, they used highly refined electrodes and small currents as compared to previous studies and used a discriminant analysis on the positive and negative electrode placements for the different types of elicited behaviour. Using these techniques, they showed that, although in overlapping patterns, a spatial organization of a number of hypothalamically induced types of behaviour exists [42,43,44].

Interestingly, different types of hypothalamically elicited behaviour appeared to be organized in longitudinal zones that to some extent correspond with the anatomical zones [42,43,44]. Digging and circling can be elicited in the lateral zone [42]. Attack behaviour was

elicited in the intermediate zone [39 40 44], flight was elicited in parts of both the intermediate and the medial zone [43] and grooming was evoked in the periventricular zone [42] (See figure 3)

In conclusion, it can be stated, that the hypothalamus is involved in the regulation of a number of behavioural responses, and that the specific parts of the hypothalamus from which these types of behaviour can be elicited, are spatially organized with extensive overlap

1.1.4.3 Behavioural circuitries involving the hypothalamus

Neuronal circuitries for the regulation of specific goal-oriented types of behaviour, such as drinking, feeding and attack behaviour, have been discussed in the literature Within these circuitries, the hypothalamus appears to play a major role in the interphase between sensory input and motor responses

Drinking as a response to hypovolemic stimuli is mediated by circulating angiotensin, acting on the subfornical organ [4 54 81 83] From the subfornical organ, efferents run to the medial preoptic area and hypothalamic paraventricular nucleus [54 81 83] From the medial preoptic area, an efferent "thirst related' (motivational) connection to the PVH and a "drinking related " (behavioural) efferent connection through the medial forebrain bundle to the brainstem have been distinghuished [81] Efferents from the PVH are suggested to play a major role in the autonomic and endocrine responses to thirst [81] Efferents from the medial preoptic area to the brain stem are suggested to be involved in the behavioural response, i e drinking [81]

The involvement of the hypothalamus in the regulation of feeding behaviour has long been studied According to the classical concept, the ventromedial hypothalamic nucleus is part of a "satiety centre" whereas the lateral hypothalamic area is part of a "hunger centre" [31] Within the hypothalamus, the dorsomedial hypothalamic nucleus (DMH) appears to be an important integrative centre of "feeding relevant' efferents from the lateral hypothalamic area (LHA) and the ventromedial hypothalamic nucleus (VMH) [46 77] The DMH projects heavily to the PVH [49 77] By the intracerebral injections of noradrenaline into the paraventricular nucleus (PVH), thus eliciting feeding behaviour, the role of the PVH in the regulation of feeding behaviour has been demonstrated [25 73] Local injections into the PVH with other substances, e g galanin and neuropeptide Y, have been found to elicit feeding behaviour as well [41 75 76] From the hypothalamus, efferent projections to other parts of the brain mediating the autonomic and behavioural aspects of the feeding response originate mainly in the PVH, but also in the LHA, DMH and VMH [47 49]

The neural circuitry underlying aggressive behaviour has been extensively studied in the cat [8 14 15 70 71 72 78] A brief overview of the brain areas involved in aggressive behaviour in the rat will be presented in chapter 3 2 In general, an aggression-facilitating pathway from the amygdala to the hypothalamic "attack" area [56 71] and an aggression- suppressive pathway, which involves the projections from the lateral septal nucleus to the mediodorsal thalamic nucleus and to the hypothalamic "aggression" area [70], have been distinghuished, although this model has already appeared too simple From the hypothalamus, the major

efferent pathway involved in aggressive behaviour seems to pass through the mesencephalic central gray [15,48,58,70,72], while an additional link to the preoptic and anterior hypothalamic areas has been suggested [56].

1.2 AIM OF THE PRESENT STUDY

With Swanson and Mogenson, we are of the opinion, that "The understanding of the neural mechanisms involved in even a single type of adaptive behaviour must be based on an understanding of the neurobiology of the central nervous system as a whole" [81]. However, to provide a full description of the intrahypothalamic neuronal mechanisms and brain circuitries involved in different types of hypothalamically elicited behaviour is beyond the aim of the present thesis.

Within the present study, emphasis will be put on the neuronal substrate underlying the behavioural differentiation of the hypothalamus, as reported by Lammers et al. [42,43,44]. Two different types of behaviour have been selected: grooming and attack behaviour. In two introductory chapters, information will be provided on the natural circumstances in which grooming and aggressive behaviour occur and what brain sites are known to be involved in the regulation of these types of behaviour (chapters 2.1 and 3.2, respectively). The results of intrahypothalamic injections of neuractive substances are presented in chapters 2.3, 2.4 and 3.4. For the study of behaviour elicited by stimulation of the ventral parts of the hypothalamus, a special cannula system has been developped and is described in chapter 3.3. The structure of grooming behaviour elicited after chemical stimulation of the hypothalamic "grooming area" is presented in chapter 2.5. The investigations on hypothalamically elicited behaviour result in the indication of behaviourally defined "grooming-" and "attack-" areas. Since the neuronal relationships are probably different, the efferent projections of the hypothalamic "grooming area" and the hypothalamic "attack area" have been investigated in a series of anatomical studies using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin and the results are presented in chapters 2.6 and 3.5. The distribution of the efferent fibres is compared with the efferent projections of other hypothalamic areas. In a general discussion, the results are summarized and discussed in view of the role of the hypothalamus and the differentiation of its efferent connections in relation to the regulation of behaviour.

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PART 2 HYPOTHALAMICALLY ELICITED GROOMING BEHAVIOUR

2.1 GENERAL INTRODUCTION TO THE STUDY OF GROOMING BEHAVIOUR

In this introduction, a review will be presented of the main characteristics of grooming behaviour. Environmental as well as intrinsic factors that alter the structure of grooming will be discussed. In addition, an outline will be presented of the brain structures, that may play a role in the modulation of grooming. Some neuroactive substances that might be involved will be discussed shortly. The emphasis will be placed on grooming behaviour of rodents, in particular of rats.

2.1.1 Occurrence and function

Grooming behaviour is clearly of great importance to rats: they spend between one third and one half of their waking time on it [13]. In non- disturbed situations grooming tends to occur after feeding and drinking behaviour. It is usually followed by "miscellaneous activity", such as exploration and sniffing. Animals usually groom before going to sleep as well [13].

The most obvious function of grooming behaviour is to keep the fur in a good condition and therefore to take care of the most important protection of the internal milieu of an animal against external challenges [67]. Grooming is also important in wound healing [37] and plays a role in the regulation of body temperature, through the spread of saliva [36,67]. Grooming behaviour may also play a role in the spreading of pheromones, produced by the harderian gland [67] and in the prevention of sexually transmitted diseases [60,38].

Grooming behaviour may occur in disturbing or frightening situations, e.g. after placing the animal in a novel environment or after the occurrence of a frightening stimulus [15,33,35,41]. This type of behaviour, i.e. behaviour that does not seem to be appropriate in a specific situation, has been called displacement activity [61,68]. Several hypotheses have been put forward to explain the occurrence of grooming in these situations. One of these relates them to an increase in non-specific arousal or emotionality [12]. However, in a novel environment, grooming behaviour appears to be dissociated from other forms of emotional behaviour, like defecation, urination and freezing [20,41]. Another hypothesis suggests that grooming in conflict situations leads to dearousal and thus forms part of a mechanism that is involved in stress regulation [19].

Both central and peripheral mechanisms appear to be involved in the execution of grooming [8,30]. Under normal circumstances, grooming appears to be under sensory control [8,31,32]. Stress-induced grooming differs in form and structure from normal grooming and the influence of peripheral sensory mechanisms appears to be smaller [8,15,31,32].

2.1.2 The structure of grooming behaviour

Grooming behaviour can be described as a series of successive behavioural elements. The structure of grooming behaviour indicates how these elements are interrelated. A set of grooming elements, bordered by non-grooming elements, forms a grooming bout. In the analysis of grooming behaviour, the study on the duration and frequency of grooming bouts, as well as on the duration of each individual element and the structure of the grooming bouts can be studied. Differences in different aspects of the structure of grooming behaviour that is elicited in various ways, either by environmental stimuli or by brain manipulation, may indicate differences in neuronal regulatory mechanisms, that have been activated.

The sequential organization of grooming under normal circumstances shows a cephalocaudal direction: the animal starts with vibrating its forepaws and continues with face washing, followed by body grooming [59,63]. In a novel environment, grooming behaviour consists of short bouts of mainly face washing [29]. When animals are exposed to a stressful stimulus (exposure to the scream of a rat threatened by a polecat), the relative amount of vibration and face washing as compared to body grooming increases, but the mean bout length of the grooming behaviour does not change [15]. This indicates, that stress-related stimuli can induce differences in the structure of grooming behaviour.

Grooming behaviour may also occur in the context of reproductive behaviour. Male rats start genital grooming after having copulated with a receptive female [38,60], while female rats lick the anogenital region during and after parturition [37].

Scratching seems to be an element of grooming that is not specifically connected to any other element of grooming behaviour [59,63]. In the sequential pattern of grooming elements, scratching is not directly built in the common (cephalocaudally directed) sequence, but it does occur either at the end or in the course of the grooming sequence. In the latter case, scratching seems to disrupt the cephalocaudal grooming sequence unpredictably [59,63].

Other indications that the structure of grooming behaviour is variable, are found in the results of studies on the behavioural effects of the central application of various neuroactive substances, as described in 2.1.3.

2.1.3 Neuroactive substances involved in grooming

Many reports have been published on the effects of intracerebroventricularly (icv) injected neuroactive substances on grooming behaviour, showing specific actions of neuroactive substances, that may elucidate some of the neural mechanisms underlying grooming behaviour. One group of neuroactive substances, the neuropeptides, has been studied in particular in its relation to grooming behaviour.

ACTH, α-MSH and ß-endorphin

Adrenocorticotrope hormone (ACTH) has received most attention, but related peptides, like α-melanocyte stimulating hormone (α-MSH) and ß-endorphin have been shown to influence some aspects of grooming behaviour as well (see below). When

injected into the brain ventricular system, ACTH increases grooming behaviour in a dose-dependent way [11,23,27,35,39,71], characterized by an increase in mean duration of the grooming bouts. The frequency of the grooming bouts and the relative contribution of each individual element in the total time spent on grooming remains the same as in control situations [35]. Besides grooming behaviour, icv injected ACTH induces the "stretching and yawning syndrome", penile erection and ejaculation and in addition affects pain regulation, memory and learning [11]. This indicates, that icv injected ACTH activates a number of different functional systems. Icv injected α-MSH induces the same behavioural response as ACTH [27,35]. Icv injections of ß-endorphin induce mainly scratching behaviour [1,65,71] and cause an increase in frequency of grooming bouts [27,35].

CRH

The results obtained by intracerebroventricular application of corticotrope releasing hormone (CRH) indicate that this peptide is involved in the behavioural responses to anxiety and stress (see 28 for review). Among these responses, an increase in grooming behaviour was found [14,26,28,54]. While icv injection of ACTH induces an increase in grooming behaviour by elongating the grooming bouts, icv injected CRH has its main effect by an increase in the frequency of the grooming bouts [26]. Scratching, however, is not increased, but shows a non-significant decrease [26].

Oxytocin, vasopressin and LHRH

Intracerebroventricular injections of oxytocin produce an increase in novelty induced grooming [21,73]. Since this increase in grooming is mainly due to an increase in genital grooming [55,73], and since injection with oxytocin induces penile erection in male rats [2a], this type of grooming may be related to sexually oriented behaviour. In this respect oxytocin differs from vasopressin, which when injected icv induces, among other behavioural effects, grooming behaviour [58], visible mainly as increases in face washing and body grooming. Injections of luteinizing hormone-releasing hormone (LHRH), a neuropeptide that is also involved in sexual behaviour, into the periaqueductal gray results also in an increase in grooming behaviour, mainly in face washing, body grooming and genital grooming [34].

Bombesin, TRH and somatostatin

Bombesin injected icv induces mainly scratching behaviour, alternated with bursts of exploratory locomotion and jerks [56,57]. Increases in anogenital grooming and paw licking have also been reported [71]. Thyrotropin-releasing hormone (TRH) and somatostatin have also been identified as scratching inducing compounds when injected icv [70,72].

Role of the pituitary

Since grooming behaviour has been viewed as a response to stress or dearousal, the role of the pituitary in novelty and peptide induced grooming, has received much attention. Jolles et al. reported that hypophysectomized rats still showed an increase in novelty induced grooming, suggesting, that this behavioural response is not dependent on an intact

hypothalamo-pituitary-adrenal axis [41]. Hypophysectomized rats still displayed excessive grooming after icv injection of ACTH as well [35,74,77]. Since the effects of ACTH on grooming behaviour and elevation of plasma corticosterone (as an effect of pituitary activation) could be dissociated, it was concluded, that the pituitary was not necessary for the novelty-induced grooming response [77]. These results were not confirmed by Dunn et al., who found an attenuation of the grooming response [23]. Destruction of the main intracerebral source of ACTH containing fibres, in and near the hypothalamic arcuate nucleus, did not result in a reduction of novelty induced grooming [24]. Therefore, it was suggested, that ACTH was released from the pituitary and transported back to the brain [24,52]. By comparing their results, Gispen and Isaacson [35] concluded, that although experimental circumstances played an important role in the amount of grooming displayed by hypophysectomized rats, grooming behaviour that is induced by stressful stimuli does not entirely depend upon activation of the hypothalamo-pituitary-adrenal axis.

In general it can be stated that different neuroactive substances show different effects upon different elements of grooming behaviour. This indicates that differences in the mechanisms may be involved in the occurrence of grooming behaviour induced by different substances.

2.1.4 Brain areas involved in grooming behaviour

Transection experiments

Transection experiments performed in cats and rats have revealed, that simple motor patterns as well as complex behaviours, such as grooming and even some kinds of habituation and associative learning can be displayed by chronic decerebrate animals [6,7]. Decerebrate rats appeared to have a larger adaptive capability than cats [6,7]. A remarkable effect of a brain transsection between diencephalon and mesencephalon was the absence of initiative to start behaviour, such as food seeking and active social interaction [6,7]. In decerebrate rats, no differences were noticed in the total number of grooming bouts per experimental session, in the amount of excessive grooming after water immersion or in the relative duration of grooming elements. In general, decerebrate animals were capable of adequately maintaining the condition of the fur [7]. After a series of descending decerebration the most notable difference was found in the gradual loss of the normal sequence of the different forepaw movements that can be indicated as vibration of the forepaws and face washing [7,10]. It was concluded, that the neuronal circuitry generating these movements is not organized in discrete centres, but consists of a diffusely organized network in the hindbrain [10]. Striatal lesions result in a disruption of the chaining of forepaw movements [9,32]. This interruption disabled the animal to continue the natural cephalocaudal sequence from face washing to body grooming, this way forcing the animal to start all over and again [9].

Electrical stimulation

Electrical stimulation has proven to be a succesful method for the elicitation of a large number of different types of behaviour, such as grooming behaviour [5,42,47]. Stimulation of brain areas situated as far caudal as the locus coeruleus and parts of the cerebellum

can elicit grooming behaviour, although in the rat the grooming behaviour elicited by electrical stimulation of brainstem sites was severely impoverished in variety and sequence of the grooming elements [3,53]. This appears to be in contrast with groooming responses elicited by electrical stimulation of brainstem areas in cats stimulation of the cerebellar region in and near the nucleus fastigii, of the parabrachial region and of the noradrenergic A5 area in the brainstem in cats elicited a well directed and sensory controlled grooming response, consisting of fur licking and fur nibbling [7] More rostrally, grooming behaviour in rats has been elicited by electrical stimulation of a hypothalamic area that included the paraventricular nucleus [47,48] In the opossum, stimulation of the medial preoptic region and of the rostral periventricular hypothalamic area elicits grooming [5] Grooming behaviour elicited by stimulation of this area was attenuated after lesions of the medial forebrain bundle, indicating a major role for this fibre system in the expression of this behavioural response [5]

A disadvantage of electrical brain stimulation is the uncertainty whether the evoked responses are due to activation of the neurons at the site of stimulation or are rather due to activation of passing fibres For the study of the morphological substrate underlying stimulation-induced behaviour this certainly is a serious disadvantage Another problem with electrical (as well as other kinds of) stimulation concerns the aftereffects, especially when (de-) arousal effects are involved, which may also play a role in the induction of grooming behaviour (see section 2 1.1) In a number of areas, including the pons [75], the septum [2], the hippocampus [49] and the lateral hypothalamus [4] (personal observation) grooming behaviour appears to be induced by cessation of the stimulation

Local chemical stimulation

The most selective method for the experimental induction of behaviour is the technique of microinjection of neuroactive substances Injection of substances into the brain ventricular system may be interesting for screening the behavioural profile of a variety of neuroactive substances (see 2 1 3), but this method does not easily permit to detect the brain structures, that are involved in the elicitation of the behaviour In an attempt to localize the site of action for the induction of grooming induced by icv injection of ACTH, Dunn and Hurd [25] closed parts of the ventricular system with cold cream They thus found, that the site of action was localized in the rostral recess of the third ventricle, near the organum vasculosum of the lamina terminalis Spruijt et al [65] reported, that the midbrain periaqueductal gray matter is neccessary for the induction of icv ACTH induced grooming, since lesioning of this brain area severely attenuated the induction of the grooming response

Local injections with neuroactive substances have shown to be the most accurate way of elucidating the possible role of brain sites in the regulation of grooming behaviour In figure 2 1, the areas, that have been reported to be involved in grooming behaviour induced by local injection of various substances and by electrical stimulation have been indicated The main areas are the mesencephalic central gray (CG) [34,46,64], the substantia nigra (SN) [62,64], the ventral tegmental area (VTA) [18,43,69] and the hypothalamic paraventricular nucleus (PVH) [40,45,46,50,51]. Other parts of the brain that have been reported to be involved in some way in grooming behaviour, are the nucleus accumbens [16,17,22,59,64,76], the neostriatum [16,17,64,76], the lateral hypothalamic area [44] and the colliculus superior [17,66].

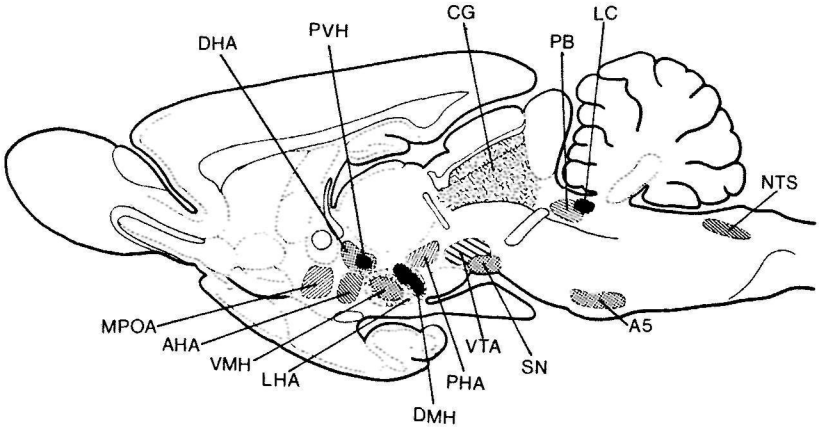

Figure 1. *Schematic sagittal drawing of the rat brain indicating different brain areas, where grooming has been elicited by local stimulation. Different hatchings are used for pictural purposes only. Abbreviations: A5: noradrenergic A5 region; AHA: anterior hypothalamic area; CG: central gray; DHA: dorsal hypothalamic area; DMH: dorsomedial hypothalamic nucleus; LC: locus coeruleus; LHA: lateral hypothalamic area; MPOA: medial preoptic area; NTS: nucleus of the solitary tract; PB: parabrachial area; PHA: posterior hypothalamic area; PVH: hypothalamic paraventricular nucleus; SN: substantia nigra; VMH: ventromedial hypothalamic nucleus; VTA: ventral tegmental area.*

It is unclear, in which way and to what extent the areas that are involved in the regulation of grooming behaviour are interconnected and able to co-operate during the proper performance of grooming behaviour, and which neuroactive substances are involved. A combination of ethopharmacology, by means of a detailed analysis of the behavioural responses elicited after local application of neuroactive substances, combined with tracing and other neuroanatomical techniques is necessary to elucidate this question.

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2.2 SOME INTRODUCTORY REMARKS ON THE HYPOTHALAMIC "GROOMING AREA"

In part 2 1, a survey has been presented of the current knowledge of neuroactive substances as well as the brain sites that may be involved in the regulation of grooming behaviour Many neuroactive substances that are known to increase grooming behaviour when injected intracerebroventricularly, like oxytocin, vasopressin and corticotrope releasing hormone (CRH), are located in neurons of the hypothalamic paraventricular nucleus [15 22] However, neurons containing some of these and other neurpeptides are not restricted to the PVH, but are also found in the adjacent dorsal hypothalamic area This holds for CRH [17 18 19], enkephalin [5 19], TRH [5 6] and to some extent oxytocin [2] Other "grooming associated" peptides, like ACTH and α-MSH are found in dense afferent projections to the hypothalamic paraventricular area (PVH) and to a lesser extent to the adjacent dorsal hypothalamic area (DHA) [10] Electrical stimulation of the PVH and DHA has been reported to elicit grooming behaviour [12] Local injections with CRH and local infusion of kainic acid have indicated, that neurons situated in this area may be involved [8 11] Taken together, a specific part of the hypothalamus, situated in and near the PVH may play an important role in the regulation of grooming behaviour Whether the induction of this response is restricted to the PVH or includes other parts of the hypothalamus bordering the PVH is the subject of the study presented in chapter 2 3 and 2 4 In chapter 2 5, a further analysis of the structure of the grooming response, as elicited by stimulation of neurons in this area is presented

The PVH is not only involved in the regulation of grooming behaviour Local stimulation of the PVH results in cardiovascular responses as well [8 16] The PVH also appears to be involved in the regulation of the energy balance [1 20] and associated feeding behaviour [13 14], in the regulation of drinking behaviour [21 23], in stress responses [7 22] and in the interaction between central nervous system and the immune system [3] In which way these functions are regulated within the PVH, whether they are working synergistically or mutually inhibiting, and whether they share the same neuronal substrate is not known at present Indications that the regulatory mechanisms of some of these responses and grooming behaviour may interact are presented in chapter 2 1 Such interactions may be present in stress-induced grooming, the occurrence of grooming and concomittant cardiovascular responses after local infusion of kainic acid into the PVH, and the occurrence of grooming after feeding [4 8 9] In chapter 2 4, some indications are presented of the involvement of the PVH in the interaction between grooming and feeding

The efferent connections of the hypothalamic area, where grooming can be elicited, as well as the specificity of these connections for the hypothalamic "grooming area" compared to other hypothalamic sites and the relation of the hypothalamic "grooming area" with other "grooming associated' brain areas will be presented in chapter 2 6

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2.3 GROOMING BEHAVIOUR ELICITED BY KAINIC ACID EVOKED CELL BODY STIMULATION IN THE HYPOTHALAMUS OF THE RAT[1]

Summary

Electrical stimulation of different parts of the rat hypothalamus has shown that the paraventricular nucleus (PVH) and dorsal hypothalamic area (DHA) form part of the neural substrate involved in grooming behaviour In order to differentiate between the possible involvement of either cell bodies or passing fibres, selective stimulation of cell bodies by low doses of kainic acid was performed throughout the rostral parts of the hypothalamus Pronounced grooming behaviour has been observed after injections into the PVH/ DHA region, indicating that neuronal cell bodies in that region are involved in the elicitation of grooming behaviour

Introduction

It has been suggested that the hypothalamus is involved in the adjustment of autonomic regulation to the requirements of behavioural responses [1] By electrical stimulation of parts of the hypothalamus a number of different behavioural responses have been evoked (see Jurgens [2] for a review) It has become clear that the behavioural response that is elicited, is strongly dependent on the hypothalamic site that is stimulated [3,4,5,6,7]. However, the borderlines of the parts of the hypothalamus involved in specific behavioural responses cannot be easily determined and areas in which different behavioural responses can be evoked, seem to overlap This may be due to the limited spatial resolution of the techniques that are used, but may also have a functional significance. Therefore, it is useful to determine the exact nature of neural elements involved in the specific behavioural responses. The aim of the present study was to further analyse the hypothalamic substrate involved in grooming behaviour

Grooming behaviour has been evoked previously by intracerebroventricular (icv) injections of a number of neuroactive substances, like ACTH [8,9,10], oxytocin [11], TRH [12], CRF [13,14], ß- endorphin [15] and bombesin [16,17] The type of grooming behaviour may differ as different substances are injected ACTH and CRF show an increase in face washing and fur grooming, while bombesin, TRH and ß-endorphin mainly induce scratching and paw licking Grooming behaviour could also be evoked by injections of either oxytocin or cholecystokinin or both into the lateral hypothalamic area [18]. This effect was suggested to arise from the oxytocin/ cholecystokinin containing neurons in the magnocellular part of the paraventricular nucleus (PVH). In a behavioural mapping study of the rostral half of the hypothalamus by electrical stimulation it appeared that grooming behaviour could only be evoked in and near the PVH [5]. Therefore, it seems likely that neuronal elements in and/

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[1] *T.A P Roeling, J.G. Veening, M.R. Kruk & R. Nieuwenhuys, Neuroscience Research Communications 6 (1990) 111-118*

or near the PVH are involved in the regulation of this behaviour. Since electrical stimulation does not allow the differentiation between the activation of fibres or neuronal cell bodies, intracerebral injections of low doses of kainic acid (KA) were used in order to stimulate cell bodies specifically, as has been described recently [19]. Injections were made throughout the rostral half of the hypothalamus of the rat, in order to localize the distribution of sites where grooming behaviour can be elicited. Using such low doses of KA lesioning of cell bodies does not seem to occur [20]. However, since higher doses of KA can be used to lesion cell bodies, it remains necessary to check for signs of neural damage.

Materials and methods

56 male Wistar rats (weight 270 - 300 g) were used in this study. Under pentobarbital anaesthesia (Narcovet, 1 ml/ kg) stainless steel guide cannulae (od. 0.4 mm, id. 0.3 mm, length 13 mm, Minitubes, Grenoble, France) were stereotaxically implanted at the initial coordinates 1.80 mm. posterior, 0.4 mm. lateral and 6.5 mm. ventral from Bregma according to [21] (flat skull position). The cannulae were kept in place by dental carboxylate cement anchored with stainless steel screws attached to the skull. Clogging was prevented by a stainless steel stylet (od. 0.27 mm) in the guide cannula. After implantation, the animals were housed individually with food and water ad lib. The animals were allowed to recover for at least one week in which they were familiarized with the experimental procedure and experimental cage: the animals were handled according to the injection procedure and placed in the experimental cage, in wich they were allowed to explore for 10 minutes. This procedure was repeated three times on different days.

The experiments were performed during the light period of the day between 9.00 and 12.00 am. During injection, the animal was held by the experimenter hand and an injection cannula (od. 0.28 mm, id. 0.18 mm) connected to a Hamilton microsyringe (1 µl) by waterfilled polyethylene tubing, as described previously [19], was lowered through the guide cannula, extending 1 mm beyond the tip of the guide cannula. A dose of 40 pmol of KA, dissolved in 0.2 µl saline (pH adjusted to 7 with 0.1 N NaOH) was injected within 30 seconds. The cannula was left in place for another 30 seconds and then quickly replaced by the stylet. The animal was put in the experimental cage (60x60x60 cm, floor covered with woodshavings, food and water ad lib.) and its behaviour scored during the next 10 minutes.

Specific elements of grooming, such as shaking, scratching, face washing, fur grooming, genital grooming, paw licking and tail grooming, were carefully recorded in order to be able to compare these data with other types of grooming behavior. These ethological data will be reported in a separate publication. For the present publication, the total time spent on grooming behaviour was taken as a general measure for the effectiveness of the injection site on grooming behaviour. If the KA injection induced an increase in grooming behaviour of more than 20% of the observation time, as compared with control situation, the injection site was classified as a "grooming-positive" site. An increase of grooming between 10% and 20% was considered an intermediate response.

The control situation consisted of a sham injection, during which the whole experimental procedure was followed except for the lowering of the injection cannula and the injection of the substance. Occasionally, control injections with saline were made in order to test for injection effects.

If a KA injection failed to induce any pronounced grooming behaviour, another KA injection was made 0.5 or 1.0 mm. deeper than the previous injection. Intervals between successive injections were always more than two days.

Upon completion of the experiment the animals were perfused with 4% paraformaldehyde/ 0.05% glutaraldehyde in 0.05 M. phosphate buffer (pH 7.4) and stored overnight in phosphate- buffered 10% sucrose solution. 40 µm frozen sections were made and stained with cresylviolet.

All injection sites were plotted in a new cytoarchitectonic atlas of the hypothalamus of the rat [22,23] and the distributions of the positive, intermediate and negative sites have been compared with the sites, which in a previous study have been found to elicit grooming behaviour by electrical stimulation [5].

Results

Following saline injections as well as sham procedures the animals showed mainly exploratory behaviour (70% of the observed time). The amount of grooming behaviour in these situations tended to be limited to less than 10 % of the observed time. No differences were seen between sham and saline control.

Following KA injections animals groomed between 0% and 80% of the observed time. Clear grooming responses were evoked in 25 sites, intermediate responses in 16 sites and no grooming responses in 25 sites. The means of each group are shown in fig. 1. The grooming behaviour evoked by KA injections contained all elements of naturally occurring grooming behaviour. In only a few animals some stretching and yawning was induced. Figure 2 shows a representative case (Rat R56), wherein grooming behaviour was increased after a KA injection.

Measurements of the distances between the guide cannulae and the tips of the injection cannulae revealed, that, using the method described, tissue was compressed by insertion of the injection cannula. As a result the effective injection site was measured to be on average 0.5 mm instead of 1.0 mm below the guide cannula. Fibre tracts, e.g. the

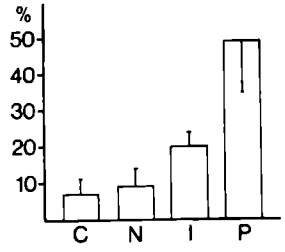

Figure 1: Time spent on grooming behaviour shown for the separate groups (see text) as a percentage of total observation time (means and S.E.). C = control; N = negative response sites; I = intermediate response sites; P = positive response sites.

fornix, in the tract of the injection cannula, reduced this distance even more. After repeated injections the distance between injection site and guide cannula tended to increase towards 1 mm, indicating the possibility of small differences in the localization of neurons activated by repeated injections.

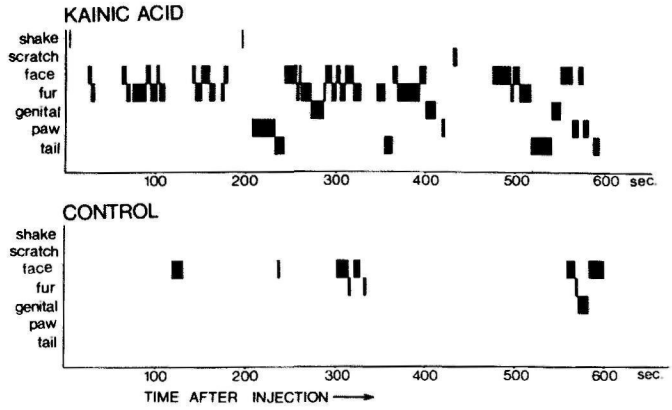

Figure 2: Time structure plot of grooming behaviour after kainic acid injection (rat no. R56). Various elements of grooming behaviour are shown in sequence of occurrence.

Histological control of the brain tissue surrounding the injection sites revealed that only a small area of gliosis between the injection site and the tip of the guide cannula is present. There was almost no gliosis observed ventral, lateral and dorsal to the injection site.

Grooming sites were mostly located in the hypothalamic paraventricular nucleus (PVH) and the adjoining dorsal hypothalamic area (DHA). They were located along the full rostrocaudal extent of the PVH (Fig 3). Few positive sites were found caudal to the PVH (Fig 3D) and one site was found at the ventral border of the anterior hypothalamic area (Fig 3B). Sites with intermediate effects were mostly found in the region near the DHA/ PVH.

Discussion

The results show, that low doses of KA can evoke grooming behaviour when injected into specific parts of the hypothalamus. Although the dose used has been reported to have no toxic effects on mesencephalic central gray neurons [19,20], we have carefully examined the injection sites for possible cell damage. Since gliosis was only found in a small area between guide cannula and injection site, we consider this to be an effect of the injection procedure rather than an effect of KA excitotoxicity. Although neuronal cell death by KA near the very centre of the injection site cannot completely be excluded, the behavioural responses can be elicited repeatedly [19] (personal observations). This suggests, that grooming behaviour was evoked by stimulation of cell bodies and not by neuronal damage. Since no excessive scratching was noticed, the action of KA on hypothalamic neurons is in some respects different from the effects of icv. injected bombesin [16], TRH [12] or ß-endorphin [15], and more similar to the effect of icv. ACTH [8,9,10,16] or CRF [13,14], but further ethological analysis is needed.

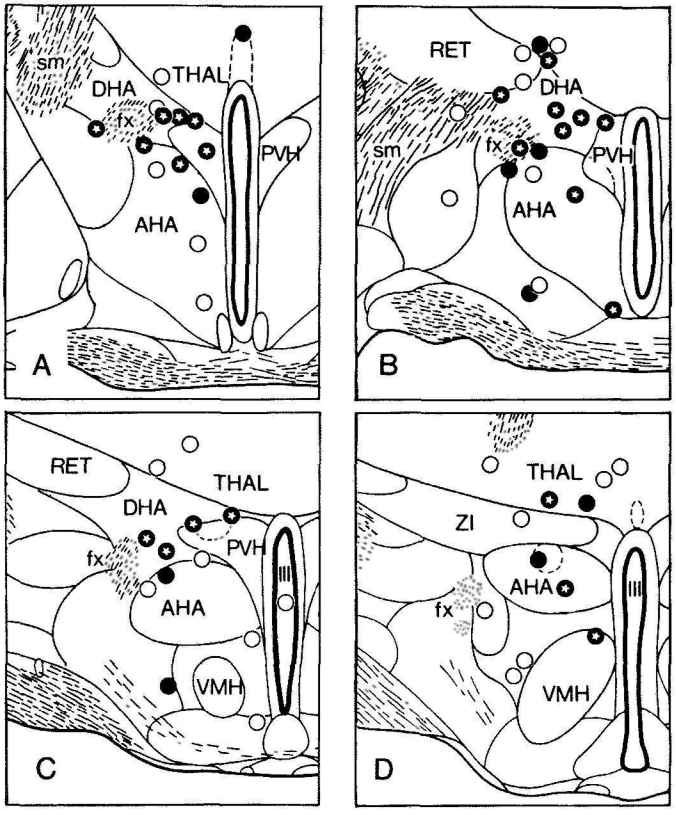

Figure 3: Distribution of injection sites on frontal planes from rostral (3A) to caudal (3D) from the atlas of Geeraedts et al. [22,23]. *Plate distance 300 µm.* ✪*:Positive sites,* ●*:intermediate sites,* ○*:negative sites. Abbreviations: AHA: anterior hypothalamic area; DHA: dorsal hypothalamic area; fx: fornix; PVH: hypothalamic paraventricular nucleus; RET: thalamic reticular nucleus; sm: stria medullaris; THAL: thalamus; VMH: hypothalamic ventromedial nucleus; ZI: zona incerta; III: third ventricle.*

The exact localization of the perikarya involved in the induction of grooming behaviour cannot be indicated as yet with absolute certainty, as long as the activated neurons themselves have not been labeled individually in some way. The lack of knowledge about the diffusion distances, as well as possible variations in the sensitivity of different types of neurons for KA complicates the relationship between the position of the injection site and the population of cell bodies responsible for the described behavioural effect. In the central gray, effective injection areas have been described to have a radius of

about 0 5 mm [19] In our experiments, differences in injection sites of 0 5 mm could make a difference between a positive and a negative response within the same animal, suggesting an effective injection radius of about 0 25 mm Since this difference occurred both in animals that first gave a grooming response as well as in animals that first gave a non-grooming response, repetition of the injection procedure itself was considered to be not important in this respect

The neurons involved in grooming behaviour appear to be located in the PVH and the adjoining DHA Their position shows a close resemblance to the area, where grooming behaviour could be elicited by electrical stimulation (fig 4) [5] Behavioural studies by means of locally applied neuroactive substances and immunocytochemical and neuronal tracing studies should further characterize these neurons and their connections with other brain sites Injections of CRF into the PVH have already been reported to induce grooming behaviour [24], although this area seems to be devoid of specific CRF- receptors [25] ACTH injections into the PVH/ DHA region are also capable of eliciting grooming behaviour [26] Many ACTH immunoreactive fibres can be found in this area [27], probably originating from the arcuate nucleus and surrounding area [28] Magnocellular PVH cells containing both oxytocin and cholecystokinin and projecting to the ventral tegmental area and the lateral hypothalamic area are suggested to be involved in grooming behaviour [19 29] However, there seems to be no clear overlap between these cells and the ACTH innervation of the PVH/ DHA area [27] Projections from the PVH to the periaqueductal gray [30] may also be important in the regulation of grooming behaviour, since this area seems to be required for the induction of grooming behaviour by icv injections of ACTH [31 32] The connections of the PVH/ DHA region and their relation to grooming behaviour are currently studied in our lab

Summarizing, it can be stated, that we have observed a rather circumscribed region in the rostral hypothalamus, including (parts) of the paraventricular nucleus and the adjoining dorsal hypothalamic area, from where activated cell bodies are able to initiate grooming behaviour In the normal activation of these cells, both CRF [24] and ACTH [26] may play a role It is not known, however, in what amount different cell types in the paraventricular nucleus are involved in grooming behaviour and whether these cells are

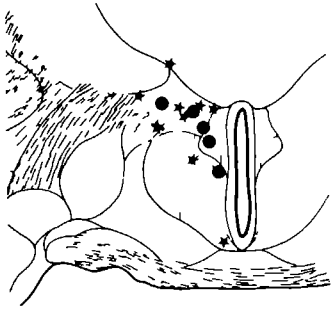

Figure 4 *Comparison of positive sites for kainic acid induced grooming (✱) with sites positive for electrically elicited grooming behaviour (●) as reported by Lammers et al [5] Frontal plane identical to figure 3B*

identical to or completely different from the neurons, that are involved in the regulation of food intake, as induced by local injections of noradrenaline [33 34] and neuropeptide Y [34 35] in the same area

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2.4 BEHAVIOURAL EFFECTS OF NMDA INJECTED INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF THE RAT[2]

Summary

Electrical stimulation of the hypothalamic paraventricular nucleus (PVH) and of the adjacent dorsal hypothalamic area (DHA) evokes grooming behaviour. Microinjections of low doses of kainic acid, an agonist of the kainate type of glutamate receptors, into the same area evokes the same behaviour. To test whether other glutamate receptors are involved, microinjections with N-methyl-D-aspartic acid (NMDA) were made into the PVH/ DHA area and the behaviour was observed. From the total observation time (30 min.) up to 73 % was spent on grooming, accompanied by yawning. Pronounced feeding behaviour was also noticed at three injection sites but not before 23 minutes after injection. Conclusions are that neurons within the PVH/ DHA area are involved in grooming behaviour, possibly via glutamatergic innervation. The interaction between grooming and feeding behaviour at the level of the PVH is discussed.

Introduction

Grooming behaviour has been described to have three separate functions: to maintain the condition of an animals fur, to regulate the body temperature and to normalize the arousal level of an animal after stressful events [13]. Studies using intracerebroventricular (icv.) injections of various peptides have indicated, that eg. ACTH [4 11], bombesin [27], TRH [34], oxytocin [3] and CRF [5 24] are involved in the neural system regulating grooming behaviour. The characteristics of the displayed grooming behaviour, however, can be different as various substances are used [11, 34, 35].

Although the neural mechanism underlying the grooming response has not yet been clarified, several brain sites have been reported to be of importance in this respect. The central gray matter [30] and the ventral tegmental area [14, 32] have been reported to be involved in peptide induced grooming behaviour. Dunn suggested, that the site of action of icv injected ACTH inducing excessive grooming was situated in the anteroventral recess of the third ventricle, near the organum vasculosum of the lamina terminalis [4]. Lammers et al. reported grooming behaviour evoked by electrical stimulation in and near the hypothalamic paraventricular nucleus (PVH) and the adjacent dorsal hypothalamic area (DHA) [18, 19]. Local injection into the PVH/DHA region of substances, that have been reported to evoke grooming behaviour by icv injection, were also capable of inducing grooming behaviour, eg. ACTH [33] and CRF [16].

Grooming could also be elicited by local intrahypothalamic injection of low, non-damaging doses of kainic acid into the PVH/ DHA region [12 28]. Kainic acid is a known agonist of the kainate type of glutamate receptors which is involved in fast synaptic neurotransmission [2]. The localized effect of kainic acid on grooming behaviour therefore

[2] *T.A.P. Roeling, A.M.M. van Erp, W. Meelis, M.R. Kruk & J.G. Veening, published in Brain Research 550 (1991) 220-224*

suggests a role of PVH/ DHA neuronal cell bodies in this type of behaviour [28].

To test whether this effect was specifically mediated by the kainic acid type of glutamate receptors, we decided to investigate the effects of other excitatory amino acids. In this report we present the results of intracerebral microinjections with N-methyl-D-aspartic acid (NMDA). NMDA is an agonist of the NMDA type of glutamate receptor which is suggested to be involved in more longlasting neuronal effects than the kainate type of glutamate receptor [2].

Since the hypothalamic paraventricular area is involved in other behaviours as well, eg. feeding behaviour [9 17 20 31] and yawning/ penile erection [1 22 23], we decided to register the complete behavioural pattern that was displayed. This enabled us to compare the results of local injection of NMDA not only in a quantitative way as far as it concerns grooming behaviour, but also in a qualitative way

Materials and Methods

The experimental design that has been used was identical to that of earlier experiments [33]. 6 male albino rats (Wistar/CPB) weighing 400-500 g were used. Under Hypnorm anaesthesia they were implanted bilaterally with 13 mm stainless steel guide cannulae (Minitubes, Grenoble, France; od 0.4mm, id. 0.3 mm.) aimed at the coordinates 7.40 mm anterior, 0.5 mm. lateral and 3.90 mm. dorsal to the interaural midline, (coördinates of Paxinos and Watson, [30]), at an angle of 10°. In this way the tip of the guide cannula was positioned at about 2 mm. dorsal to the PVH. A stainless steel stylet was placed into the cannula to prevent it from clogging. After surgery the animals were individually housed in home cages (50x50x50 cm) with free access to food and water and handled for one week.

The experiments were performed between 13.00 and 17.30 hr. p.m Animals were injected on the left side or right side with NMDA (Sigma, 0.2nmol in 0.2 µl saline, pH adjusted to 7 with 0 1 N NaOH) or sham injected in a randomized order with 48 hours between each session. The injection procedure has been described in earlier reports [28 33]. In short, an injection cannula (od. 0.28 mm, id 0.18 mm), connected to a 1 µl Hamilton microsyringe via waterfilled polyethylene tubing, was inserted, extending 2 mm beyond the tip of the guide cannula. The NMDA solution was injected within 30 seconds and after an additional 30 seconds the injection cannula was quickly replaced by the stylet and the animal was placed back in its home cage. A video camera connected via a video timeframe encoder/ decoder (part of a new data analysis system, PC-protocol, developed at the Ethopharmacology department and produced by IEC-ProGamma, Groningen, The Netherlands) to a video recorder, was placed in front of the glass front wall of the cage and video recordings of the rats behaviour were made for a period of 30 minutes.

After completion of the testing the tapes were analysed using PC-protocol. For the present report, the total time spent on grooming behaviour is used for description of the effects of NMDA on this behaviour. The following elements were considered to be part of grooming behaviour: Vibration of the forepaws, face washing, fur grooming, tail licking, hindpaw licking, anogenital licking, scratching and shaking. Other behavioural elements, that were observed and denotated separately, were yawning, feeding/ drinking and resting/ sleeping.

After completion of the series of experiments, the animals were deeply anaesthetized with ether and transcardially perfused with 4 % paraformaldehyde and stored

overnight in 0.1 M. phosphate buffer / 20 % sucrose, pH 7.6. Frozen sections (40 µm.) of the injection sites were cut and counterstained with Giemsa staining [10]. The injection sites were plotted in a detailed cytoarchitectonic atlas [6, 7].

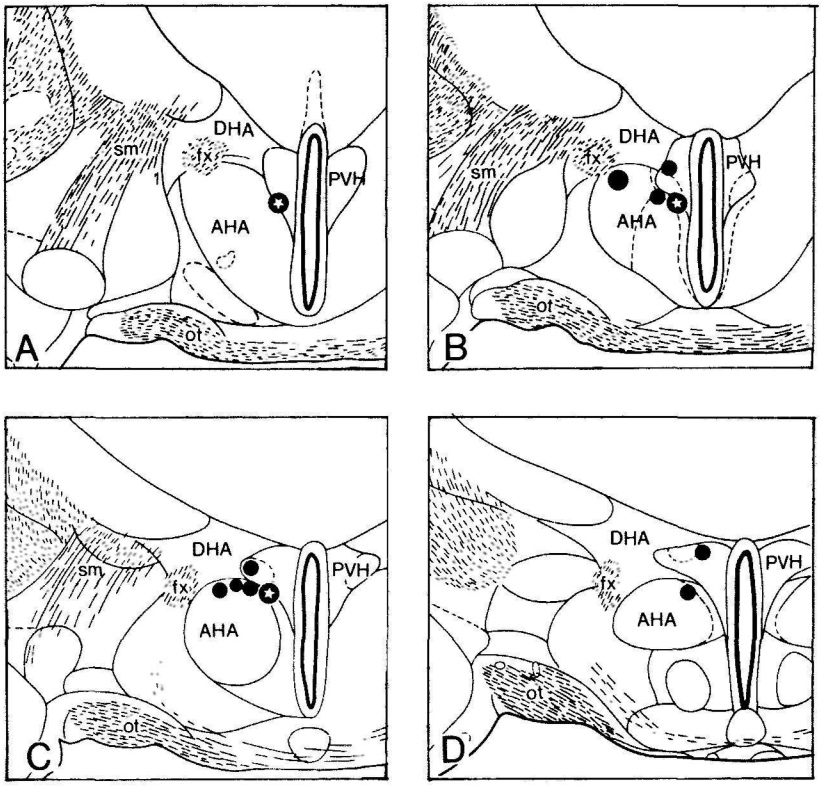

Figure 1: *Injection sites plotted on the atlas of Geeraedts et al. [6, 7]. Plates are indicated from rostral (A) to caudal (D) with intermediate distances of 150 µm. The size of the black dot is an indication of the amount of grooming behaviour that was elicited with the largest dots indicating sites where the total amount of grooming exceeded 50 % of the observation time. Injection site with the largest effect (73% of observation time) is pointed out with an arrow. Black dots with star indicate sites where feeding was also elicited (see Text). Abbreviations: AHA = anterior hypothalamic area; DHA = dorsal hypothalamic area; fx = fornix; ot = optic tract; PVH = paraventricular nucleus; sm = stria medullaris.*

Results

The deepest point of the cannula tracts where some gliosis could be observed, was plotted on the atlas (Fig.1). Four sites were located exactly within the paraventricular nucleus. Six tracts passed the paraventricular nucleus and extended just below the ventrolateral border of the PVH and two sites were located in the dorsal part of the anterior hypothalamic area, ventral to the dorsal hypothalamic area.

The total amount of grooming was significantly increased in all cases (47.0 ± 4.0 %, control 18.9 ± 4.8 % of total observed time) with the highest response being 73% of the observation time spent on grooming (Wilcoxon matched pairs rank test, $p<0.05$)(Fig. 2a,b). After NMDA injection, the animals started to groom immediately after replacement

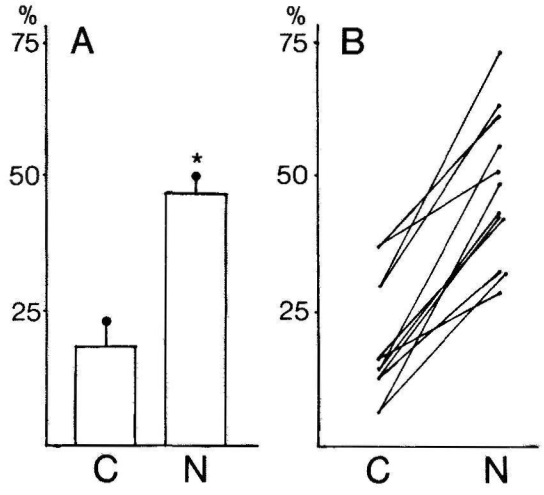

Figure 2: Grooming behaviour elicited after NMDA injection into the PVH/DHA area (N) as compared to control situation (C). a) Mean ± S.E.M. indicated as percentage of total observed time. b) Individual responses as a percentage of total observed time. n = 6 for control, n = 12 for injection sites.

into the homecage. The amount of grooming behaviour as measured within five minute periods gradually declined, but remained higher than control levels during the entire observation time of 30 minutes (Fig.3). In the control situations, the animals started some grooming after a short exploration of the cage and in most cases they slept during the last 5-10 minutes of the observation time. Grooming behaviour induced by NMDA showed all elements of naturally occurring grooming behaviour (vibration of the forepaws, face washing, fur grooming, anogenital grooming, tail licking, scratching and shaking). No excessive amount of any of these elements as compared to the amount of the other elements of grooming behaviour was seen.

In most cases an increase in yawning was seen (frequency 8.9 ± 1.5, control 1.5 ± 0.7), frequently, but not always, accompanied by stretching. However, a clear clustering of injection sites that gave the highest frequency in yawning responses within the total area that was covered by the injection sites could not be noticed.

At three most medially situated injection sites, a considerable amount of feeding

behaviour was elicited, that consisted of more than 10 % of the total observation time (Fig. 1). This behaviour consisted of the full repertoire of grasping food out of the storage box, using the upper and lower incisors, gnawing on the pellets, using the forepaws and chewing, alternated with drinking. This behaviour was not seen until about 23 minutes after the beginning of the observation time. Since this amount of feeding behaviour was never seen in the control situation, we tend to consider it as a late- appearing effect of the NMDA injection. In these sites grooming behaviour was also greatly increased and preceeded the feeding response (Fig 1).

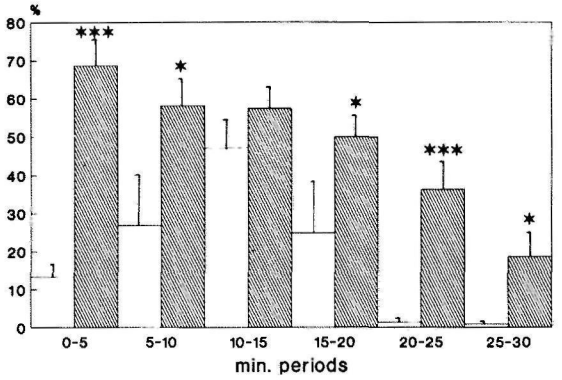

Figure 3: *Percentage of time spent on grooming behaviour as indicated for five minute periods after injection. Open bars are control values, filled bars are experimental values. Mean values ± S.E.M. for n = 6 (controls) and n = 12 (experimental group). * and *** indicate significancy from control for p<0.05 and p<0.001 respectively (2-way ANOVA).*

Discussion

The injection sites as shown in figure 1 are all in such close proximity to the area indicated in earlier reports [18, 28, 33], that we consider the injection sites to be located within the "grooming area".

By injecting NMDA into the hypothalamic paraventricular nucleus (PVH) and adjacent dorsal hypothalamic area (DHA), we induced an increase in total amount of grooming behaviour up to 73% of the total observation time of 30 minutes (Fig 2b). In a previous report, we have shown that microinjections of kainic acid (KA) evoked the same response [28]. Taken together, the results strongly suggest, that neuronal cell bodies in this area are responsible for the observed behavioural effect and that a direct involvement of glutamate- like activity seems evident.

The results described in the present report on grooming behaviour are comparable with the results of local injections of other substances into this region. CRF and $ACTH_{1-24}$ are also capable of inducing grooming behaviour when injected into the PVH/ DHA region [16, 33]. However, there have been no indications for the presence of receptors of either of these substances in the PVH/ DHA region. Therefore the explanation of the localized effects of these substances upon grooming behaviour is difficult. Since differences in the structure of the grooming responses might implicate differences in neuronal circuitry that is activated, a comparison of the characteristics of grooming behaviour elicited by both NMDA and ACTH into the PVH/ DHA area might be useful in this respect, as well as a closer study on the (co-) localization of both glutamatergic and ACTH- immunoreactive fibres within this area.

The elicitation of yawning and penile erection has been reported to be mediated by oxytocinergic neurons in the paraventricular nucleus [1, 22]. Whether this response is organized in the PVH independently from grooming behaviour or must be regarded as part of PVH induced grooming behaviour is as yet unknown. Van Erp et al. have already reported, that grooming with yawning was elicited only after ACTH injection into the PVH and while after injection into the adjacent DHA region only grooming could be elicited [33].

Pronounced feeding behaviour was observed only after injections into the most medial sites in the PVH and with a long latency. Feeding behaviour is well known as a behaviour that can be induced by injections of noradrenaline in this area [20]. This effect was suggested to be induced by inhibition of the spontaneous activity of PVH neurons, mediated through postsynaptic α_2-receptors [9, 15, 29]. Feeding behaviour after NMDA injection was seen only after about 23 minutes observation time in which the rats groomed most of the time. Intracellular currents evoked by NMDA stimulation have been reported to fade out in *in vitro* experiments [21]. It is well possible that such a decrease in activity was apparent in our experiments, although the time latency of the effects differed (150 sec. *in vitro*, 23 min. *in vivo*). This decrease in local neuronal activity may have been effective in starting the feeding response. Whether or not these neurons have been involved in the grooming response as well is still unknown. The seemingly close connection between grooming and feeding is illustrated by the observations that icv injections of several substances increase grooming while decreasing feeding, eg. CRF [16, 24], ACTH [36] and bombesin [26]. Local injections of bombesin into various brain areas, among which the PVH, showed the same effect [17]. These opposite effects were found to be specific and not merely a shift in time expenditure [36]. Such a correlation could very well be explained by the involvement of at least partially the same neuronal substrate for both behavioural systems. At the level of the PVH this could be a single population of neurons, the excitation of which results in grooming behaviour and the inhibition of which results in feeding. More conclusive evidence is needed to settle this issue.

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2.5 ANALYSIS OF GROOMING BEHAVIOUR ELICITED BY NMDA INJECTION INTO THE HYPOTHALAMUS OF THE RAT[3]

Summary

Electrical and chemical stimulation of a hypothalamic area including the nucleus paraventricularis and adjacent dorsal hypothalamic area have been reported to increase the total amount of grooming behaviour. In the present report a further analysis is made of the grooming behaviour that was elicited by local injection of N- methyl- D- aspartate (NMDA, 0.2 nmol in 0.2 µl saline). Since it appeared, that saline injections in specific sites of this hypothalamic "grooming area" were also capable to induce some grooming behaviour and since the effects on grooming behaviour are dependent on the precise location of the injection, the effects of NMDA injections in 5 specific sites were compared pairwise with 5 matched identical saline injection sites, selected from a large series of saline injections on the basis of careful histological mapping. Our results show that the total amount of grooming behaviour was increased in both saline and NMDA injection groups as compared to "handling control". Animals injected with NMDA exceeded the saline group. Differences between NMDA and saline injected animals were found in the frequencies and durations of the different elements of grooming behaviour. Higher frequencies in most grooming elements were found in the NMDA group as compared to the saline group. Another specific effect of NMDA was observed in the increase of both the frequency and the duration of genital grooming and the elicitation of yawning. Scratching was affected neither by saline- nor by NMDA injections. The present report shows that even though gross behavioural measures may seem similar, the detailed analysis of constituent parts and structure of the behaviour do reveal striking differences in the effects of different experimental conditions, stressing the importance of a close examination of the behavioural records.

Introduction

Grooming behaviour has been reported to have different functions, like temperature regulation [22], maintenance of the condition of the fur and normalising arousal levels after stressful events [11]. The structure of grooming behaviour elicited in different circumstances may differ. In a novel environment grooming behaviour has been reported to consist of short bouts of mainly face washing [6]. When rats are exposed to stressful stimuli, the relative amounts of vibrating the forepaws and face washing increase while the mean length of the grooming bouts does not change [3]. After copulation, male rats start grooming that mainly consists of genital grooming [20]. Other indications, that the structure of the grooming behaviour or single grooming elements can be changed in duration or frequency are found in studies on behavioural effects of intracerebroventricular (icv.) injection of

[3] *T.A.P. Roeling, A.M.M. van Erp, M.R. Kruk & J.G. Veening*

various neuroactive peptides. Icv. injection of ACTH increases mean duration of the grooming bouts, while the frequency of the bouts remains unchanged [10]. Icv. injection of corticotrope releasing hormone (CRH) does not change bout duration but increases the frequency of the grooming bouts [5]; Icv. injection of oxytocin increases novelty induced grooming by an increase in genital grooming [4,26], while bombesin injection induces mainly scratching behaviour [16].

Local cerebral stimulation studies have shown, that a close examination of the behavioural record is necessary. Stimulation of a hypothalamic area including parts of the paraventricular nucleus with electrical stimulation or local injection with different neuroactive substances, like neuropeptides and excitatory amino acids, have been reported to increase the amount of grooming behaviour [13,18,19,23]. The structure of the behaviour differs as different stimulation techniques are applied [23]. Moreover, latency, form and persistence of the response depend on the precise localization of the injection sites [24]. In a study on the neuronal mechanisms involved in the regulation of grooming behaviour, it is therefore necessary to look at the details of the behaviour, that is elicited.

In a previous report we presented the results of NMDA injections into the PVH/DHA region in terms of total duration of grooming [19]. For a better understanding of the behavioural effects of NMDA injections into the hypothalamic "grooming area" a closer analysis of the grooming response is required. Since at the centre of the hypothalamic "grooming area" an area has been found where even the injection of saline can cause some grooming behaviour, possibly by the release of endogenous substances [25], the contribution of NMDA itself to the behavioural change needed further study. To assess this contribution of NMDA we decided to perform a detailed analysis of the behavioural differences between pairs of specific sites which were injected with either saline or NMDA and were matched for precise anatomical localization. To avoid differences in behavioural responses due to repeated injections, the saline injections were made in a separate group. For comparison, we chose those sites from a large group of 43 saline injections [25] as close as possible to the NMDA injection sites. Handling controls from the NMDA animals were taken as a third group.

Materials and methods

Male Wistar rats (400-500 g) were cannulated with stainless steel cannulae (od. 0.4 mm, id. 0.3 mm) as reported earlier [18,19,23]. After surgery, the animals were housed in home cages (50x50x50 cm) with free access to food and water. The animals were allowed to recover for 1 week, in which they were handled. Animals in the NMDA group were injected in the left or the right side with NMDA (Sigma, 0.2 nmol in 0.2 µl saline, pH adjusted to 7 with 0.1 M NaOH) or handled (HANDLING group) in a balanced randomized order with 48 hours between each session. Animals that were used for the saline injections (0.2 µl) were prepared in the same way as the NMDA group.

Injections were made with an injection cannula (od. 0.28 mm, id. 0.18 mm) that extended 2 mm beyond the tip of the guide cannula. The injections were made within 30 seconds after which the cannula was left in place for another 30 seconds. After that the injection cannula was replaced by the stainless steel stylet which did not extend beyond the guide cannula and the animal was returned to its home cage. The handling procedure consisted of holding the animal the way it was held during injection and removing and

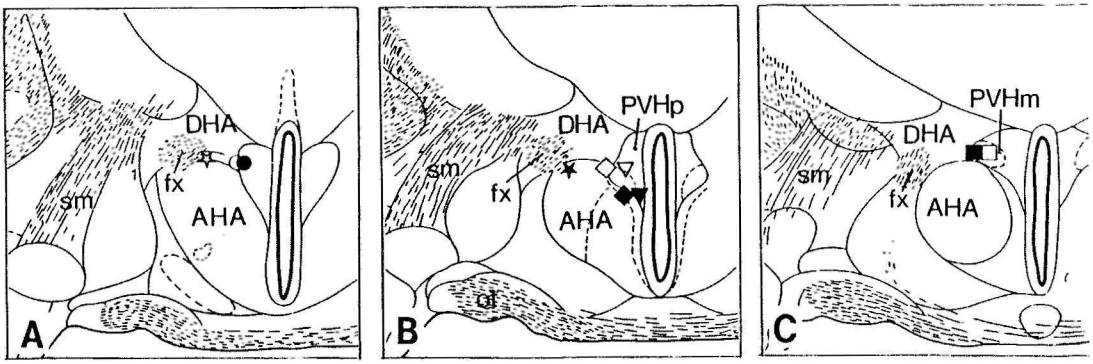

Figure 1: Injection sites plotted on the atlas of Geeraedts et al. [8,9]. Sections are indicated from rostral to caudal with intermediate distances of 150 µm. Black signs indicate NMDA injections, open signs indicate saline injections. Matched pairs are indicated by the shape of the sign. PVHp, parvocellular part of the hypothalamic paraventricular nucleus; PVHm, magnocellular part of the paraventricular nucleus; DHA, dorsal hypothalamic area; AHA, anterior hypothalamic area; fx, fornix; sm, stria medullaris; ot, optic tract.

replacing the stylet. The handling procedure lasted as long as the injection procedure. Video recordings were made during 30 minutes postinjection using a video camera connected to a new video analysis system (CAMERA), that is now produced by IEC ProGamma, Groningen, The Netherlands.

The animals were deeply anaesthetized after completion of the testing and transcardially perfused with saline and subsequent fixative (4% paraformaldehyde/ 0.05 % glutaraldehyde in 0.1 M phosphate buffered saline, pH 7.6). Brains were stored overnight in a 20 % sucrose solution. Freeze sections (40 µm) of the injection sites were cut and counterstained with cresylviolet or Giemsa staining. The injection sites were plotted in the atlas of Geeraedts et al. [8,9].

For the comparison between the NMDA and saline group, matched pairs were made on the basis of their injection sites. If no perfect matches could be determined, saline injection sites that were closest to NMDA injection sites within the same hypothalamic area were considered to be the best match.

Video recordings of the behavioural response of the selected animals were analysed using CAMERA. The following elements were encoded: vibration of the forepaws (VIBRATE), face washing (FACE), body grooming (BODY), hindpaw licking (PAW), tail grooming (TAIL), anogenital grooming (GENITAL), scratching (SCRATCH), and short breaks within grooming bouts (PAUSE). Body shakes (SHAKE) and yawning (YAWN) were also registered. A grooming bout was defined as a series of grooming elements

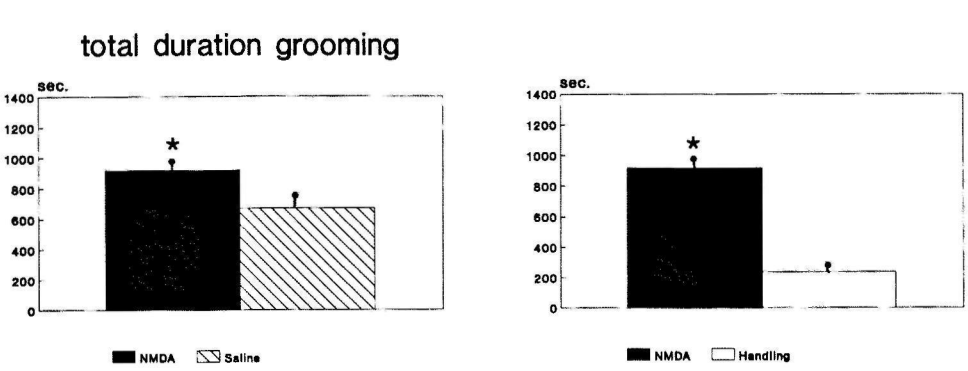

Figure 2: Total duration of grooming after injection of NMDA and saline into the PVH/ DHA area and after handling control. Mean ± S.E.M. (*): $p < 0.05$ Wilcoxon Matched pairs rank test.

preceded and succeeded by behavioural non- grooming elements. The grooming element immediately preceding shaking or yawning was found to be usually identical to the grooming element immediately following this act. We believe, that these elements are superimposed upon the ongoing grooming behaviour. Shakes and yawns preceded and followed by grooming elements were therefore considered to be part of the ongoing grooming bout.

For statistical analysis, the Wilcoxon matched pairs rank test was used for comparison between NMDA and HANDLING and between NMDA and matched saline.

Results

In the comparison between NMDA and saline injection sites, 5 matched pairs could be formed. In Fig. 1, the injection sites used for the "matched pair analysis" have been plotted in a detailed hypothalamic atlas [8,9]. All injection sites are positioned within the hypothalamic "grooming area" [18].

During handling control sessions, the animals first displayed some exploratory behaviour. After 5-10 minutes, they started to groom for a period of about 10 minutes. Animals injected with NMDA or saline started grooming almost immediately after replacement in the home cage. The grooming behaviour displayed in these groups lasted for about 20 minutes. After this period, the grooming gradually stopped.

In figure 2, the total durations of grooming behaviour after NMDA and saline injections and handling control are presented. The total amount of time spent on grooming behaviour was larger after saline injection as compared with handling control. The total duration of grooming displayed in the NMDA group was significantly increased in

comparison with the handling control and significantly exceeded the amount of grooming in the saline group as well. The frequency and the mean duration of the grooming bouts was increased in NMDA as compared to handling control (Fig.3, table 3). A small, but statistically not significant increase was noted as compared to the saline group (Fig.3, Table 3).

Inside the grooming bouts, the frequency, total and mean duration of the grooming elements showed marked differences between the NMDA and SAL groups (Tables 1, 2 and 3).

The frequency of most grooming elements (viz. VIBRATE, FACE, BODY, GENITAL, PAW, TAIL, PAUSE) was increased after NMDA injection as compared to handling control. In comparison with the matched saline injections, the frequencies of elements VIBRATE, FACE, BODY and GENITAL were significantly higher in the NMDA group (Table 1).

The total duration of a number of elements (VIBRATE, FACE, BODY, GENITAL, PAW and TAIL) was also increased when compared to handling control (Table 2). Comparing the NMDA group with the saline group significant differences were found in VIBRATE and GENITAL. Face washing was the only element that showed a significant decrease in mean duration as compared to saline treatment and also as compared to handling controls. The mean duration of body grooming showed a non-significant decrease

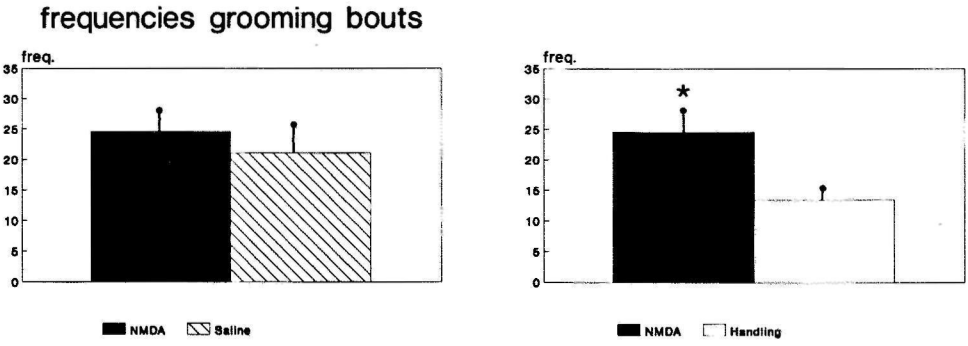

Figure 3: *Frequency of the grooming bouts after injection of NMDA and saline into the hypothalamic "grooming area" and after handling control. Mean ± S.E.M. (*): $p < 0.05$ Wilcoxon Matched pairs rank test.*

in the NMDA group as compared to the SAL group and the handling group (Table 3).

Interestingly, no changes in frequency, total duration and mean duration were found in scratching between the NMDA- and handling groups and between the NMDA-

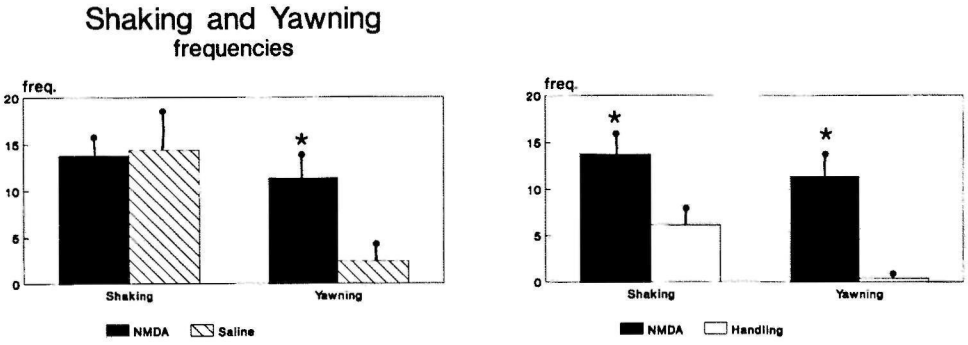

Figure 4: *Frequencies of shaking and yawning after injection of NMDA and saline into the hypothalamic "grooming area" and after handling control. Mean ± S.E.M. (*): $p < 0.05$ Wilcoxon matched pairs rank test.*

and SAL groups (tables 1, 2 and 3).

Shaking and yawning always seem to have a fixed and short duration and therefore we only registered their frequencies (Fig. 4). The number of shakes was significantly increased in the NMDA group as compared to the handling group, but no difference was found between the number of shakes in the NMDA and saline groups. NMDA gave a sizable and significant increase in yawning as compared to the handling group. This increase significantly differed from the SAL group as well.

Discussion

The present report confirms the finding that saline injections in specific parts of the hypothalamic "grooming area", may also elicit grooming behaviour [25]. NMDA injections in the hypothalamic "grooming area" increase the time spent on grooming behaviour as well [19]. The comparison between the effects of saline injections and NMDA injections on grooming behaviour is therefore important to understand the effects of NMDA itself.

The increase in total time spent on grooming after NMDA injections was significantly larger than after saline injections, due to the fact that the mean duration and the frequency of the grooming bouts were slightly higher (Fig. 2 and 3, Table 3). This could imply that NMDA either increases the effect of saline or elicits grooming behaviour specifically by activating neurons via specific receptors. The experimental setup did not allow to investigate whether NMDA itself is capable of eliciting grooming when it is applied to the hypothalamic "grooming area". Infusion of NMDA into the "grooming area" in freely moving animals is needed to elucidate this issue.

In addition to our observation that NMDA induces a larger increase in the duration of grooming behaviour than saline injections, we observed differences in the structure of the grooming bouts. A striking difference was found in the increase in frequency of

Table 1

FREQUENCIES OF GROOMING ELEMENTS

	NMDA	Saline	Handling
Vibrate	4.8 ± 1.5 ¤ *	1.2 ± 0.5	0.8 ± 0.2
Face	41 ± 3.4 ¤ *	20.6 ± 2.7	5.2 ± 0.7
Body	29.2 ± 2.0 ¤ *	17.4 ± 3.0	6.2 ± 1.3
Genital	14.6 ± 1.0 ¤ *	4.4 ± 1.0	4.2 ± 0.8
Paw	6.2 ± 1.0 ¤	7.0 ± 1.8	1.0 ± 0.3
Tail	4.2 ± 0.8 ¤	2.0 ± 0.9	0
Pause	19.8 ± 3.5 ¤ *	6.6 ± 1.2	6.8 ± 2.0
Scratch	10.0 ± 1.8	10.2 ± 2.1	10.8 ± 2.6

Table 1 Frequencies of grooming elements after injection of NMDA into the hypothalamic "grooming area" as compared to the results of saline injections and of handling control. Mean ± S.E.M. Significant differences between NMDA and saline: (*); Significant differences between NMDA and handling control: (¤). $p < 0.05$ Wilcoxon Matched pairs rank test.

Table 2

TOTAL DURATIONS OF GROOMING ELEMENTS

	NMDA	Saline	Handling
Vibrate	2.5 ± 0.8 ¤ *	0.6 ± 0.3	0.2 ± 0.1
Face	268.0 ± 21.8 ¤	210.6 ± 26.6	77.1 ± 27.0
Body	275.7 ± 10.3 ¤	223.6 ± 54.9	85.4 ± 18.4
Genital	188.6 ± 15.1 ¤ *	50.2 ± 7.3	29.5 ± 6.2
Paw	115.4 ± 22.8 ¤	138.7 ± 22.9	26.5 ± 18.3
Tail	40.6 ± 6.7 ¤	35.9 ± 16.5	0
Pause	28.4 ± 6.1	10.7 ± 2.8	14.4 ± 4.3
Scratch	144.6 ± 55.3	158.8 ± 23.9	184.5 ± 57.9

Table 2: Total durations of grooming elements after injection of NMDA into the hypothalamic "grooming area" as compared to the results of saline injections and of handling control. Mean ± S.E.M. Significant differences between NMDA and saline: (*); Significant differences between NMDA and handling control: (¤). $p < 0.05$ Wilcoxon Matched pairs rank test.

different grooming elements (table 1). The frequencies of the grooming elements VIBRATE, FACE, BODY, GENITAL and PAUSE were significantly increased as compared to saline injected animals. The total durations of VIBRATE and GENITAL were increased accordingly. The mean durations of the grooming elements did not change much, but the mean durations of face washing and, to a lesser extend body grooming were decreased. In addition, NMDA increased the frequency of yawning. Scratching was not affected by NMDA. Since it is still unknown in what way saline is capable of eliciting grooming

Table 3

MEAN DURATIONS OF GROOMING BOUTS AND GROOMING ELEMENTS

	NMDA	Saline	Handling
Grooming bouts	39.98 ± 0.85 ✿	33.80 ± 4.26	17.24 ± 2.55
Vibrate	0.41 ± 0.05	0.40 ± 0.15	0.23 ± 0.06
Face	6.61 ± 0.45 *	10.72 ± 1.61	12.98 ± 3.46
Body	9.56 ± 0.52	12.58 ± 1.58	13.97 ± 2.45
Genital	12.00 ± 0.52	13.63 ± 2.46	7.14 ± 1.86
Paw	21.68 ± 6.51	22.07 ± 2.45	24.88 ± 18.56
Tail	10.04 ± 1.40	27.67 ± 18.10	0
Pause	1.34 ± 0.11 ✿	1.56 ± 0.14	3.08 ± 1.12
Scratch	15.06 ± 4.32	18.40 ± 4.62	17.41 ± 3.37

Table 3: Mean durations of grooming bouts and grooming elements after injection of NMDA into the hypothalamic "grooming area" as compared to the results of saline injections and of handling control. Mean ± S.E.M. Significant differences between NMDA and saline: (*); Significant differences between NMDA and handling control: (✿). $p < 0.05$ Wilcoxon Matched pairs rank test.

behaviour, it is difficult to understand the way in which NMDA is capable of changing the structure of grooming behaviour. Several mechanisms may be involved in this respect.

The increase in pauses as presented in this report, was also found with electrical stimulation of the same hypothalamic area [23]. This increase was suggested to arise partly by the non-selectiveness of electrical stimulation, possibly evoking competing and incompatible responses simultaneously [23]. On the other hand, the increased pause frequency induced by NMDA may be the behavioural expression of a stressor mechanism. As a result it induces the animal to scan its environment to make sure that it is safe to continue with body care. It is therefore conceivable that a major effect of NMDA is that it causes an increase in pause frequency, thus interrupting the completion of the generally rostrocaudal sequence of grooming, forcing the animal to start the sequence over and again at the rostral beginning. The increase in frequency of the elements VIBRATE, FACE and BODY, which elements are known to occur at the beginning of a grooming bout [2,7,17], may be the result of such a mechanism.

The increases in the frequencies of yawning and genital grooming is in accordance with other reports on behavioural effects of manipulation of the PVH. Injections of oxytocin and dopamine agonists into the PVH have been reported to elicit penile erections and yawning [1,14,15]. This response was suggested to arise from a direct activation of oxytocinergic neurons in the PVH [14,23]. Oxytocin has been reported to induce grooming behaviour when injected in other brain sites as well [12,21]. When injected intracerebroventricularly, oxytocin induces grooming behaviour as well and specifically genital grooming [26]. The hypothalamic "grooming area" is rich in oxytocin, and the projections of the grooming area are very similar to the distribution of oxytocin immunoreactivity in the brain (Roeling et al., submitted). Moreover, oxytocin is able to induce grooming following i.c.v.-and PVH-infusion in resting animals [25]. Therefore, one is tempted to suggest that

NMDA might act via oxytocin-containing neurons in inducing yawning and genital grooming.

Interestingly, scratching, a body care response often associated with grooming, is not affected by any of the experimental conditions. This supports earlier observations [23] suggesting that at the level of the PVH grooming and scratching are controlled by different neural mechanisms. However, manipulation of the "grooming area" may affect scratching, since electrical stimulation reduces the amount of scratching, that is displayed [23]. Low doses of kainic acid, which have been reported to elicit grooming behaviour when injected into the "grooming area" area [18], reduce scratching behaviour as well (Roeling, unpublished results).

The results of the present report show that general measures of behaviour are insufficient to describe behavioural effects. Intracerebral manipulations may change the structure of behaviour profoundly while leaving the overall measures unaffected. In addition, the importance of a detailed histology is important for further study of the mechanisms that are involved in the regulation of behaviour. The present report clearly shows that NMDA does not only enhance the effect of saline injections on grooming behaviour, but also changes the structure of the grooming behaviour.

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2.6 EFFERENT CONNECTIONS OF THE HYPOTHALAMIC "GROOMING AREA" IN THE RAT[4]

Abstract

The efferent connections of the hypothalamic area, where grooming can be elicited by local electrical stimulation or injection of various substances, were studied using iontophoretic injections of *Phaseolus vulgaris* leucoagglutinin. This hypothalamic "grooming area" (HGA) consists parts of the hypothalamic paraventricular nucleus and of the dorsal hypothalamic area. The specificity of these efferents for the HGA was investigated by comparison with efferents of hypothalamic sites adjacent to this area. In addition, the distribution of oxytocinergic fibres was studied, since oxytocinergic neurons are present in the HGA and oxytocin is possibly involved in grooming behaviour.

The efferents of the HGA as well as of hypothalamic sites surrounding the HGA and the oxytocinergic fibres studied do not form well determined bundles, but rather spread out throughout the hypothalamus. Clusters of fibres could be traced rostrally and caudally, forming diffuse fibre "streams". Three rostral, two thalamic and three caudal fibre "streams" have been distinghuished along which efferent fibres innervate different brain areas. The many varicosities on labelled fibres "en passant" suggest that hypothalamic fibres are able to influence many parts of the brain along their way.

The anterior periventricular area, the median preoptic nucleus, the ventral tegmental area and nucleus of the solitary tract were found to be more or less specifically innervated by HGA fibres and oxytocinergic fibres. Other brain areas, like the septum, the medial amygdaloid nucleus, the central gray and the paraventricular nucleus of the thalamus were found to receive efferent projections from the HGA and hypothalamic loci outside the HGA, as well as from the oxytocinergic system. Within the septum and the mesencephalic central gray, differences in the spatial organisation of terminating fibres from HGA and non-HGA sites have been found. Fibres from the HGA clustered in the ventral part of the lateral septal nucleus, while fibres from surrounding hypothalamic loci innervated other parts of that brain area. In the cental gray, fibres from the HGA clustered in rostrodorsal and caudoventral parts of the central gray. A number of brain areas, that are innervated by HGA fibres and oxytocinergic fibres, like central gray, ventral tegmental area and the noradrenergic A5 area, have been reported previously to be involved in grooming behaviour.

It is concluded from the present findings, that the hypothalamic "grooming area" has preferential connections with a number of brain sites, not shared with hypothalamic projections from outside the "grooming area". Some of these destinations are known to be involved in grooming behaviour. The unravelling of these behaviourally relevant circuitries, together with the characterization of its connections and the description of the be-

[4] *T.A.P. Roeling, J.G. Veening, J.P.W. Peters, M.E.J. Vermelis & R. Nieuwenhuys, submitted*

havioural responses elicited by stimulation of parts of this circuitry, is important in the study on brain mechanisms that are involved in the regulation of behaviour.

Introduction

The hypothalamus plays an important role in the integration of behaviours with associated autonomic and endocrine responses [97]. By using electrical stimulation of different parts of the hypothalamus, it has been demonstrated, that a large number of behaviours can be elicited, like locomotion, feeding, drinking, aggression, digging and grooming [36,42,44,45,47,48,49,66,106]. Although there has been a controversy in literature on the nature of the elicited response and the site-specificity of hypothalamically elicited behaviours [17,78,86,99] there is now convincing evidence, that the location of the stimulated hypothalamic site is an important determinant of the type of behaviour, that is elicited. By using a refined stimulation technique, combined with a sophisticated discriminant analysis on a large number of electrode placements, Lammers et al. have shown, that within the hypothalamus there is a spatial organization in the distribution of different types of stimulation-induced behaviour [47,48,49].

The anatomical basis underlying this behavioural specificity of the hypothalamus is still largely unknown. However, it is conceivable, that different types of hypothalamically elicited behaviour require a differential activation of other parts of the brain. In addition, different kinds of behaviour require different levels of autonomic activity in a variety of organs. Therefore, sites where a specific type of behaviour can be elicited should have different efferent connections as compared to other behaviourally determined hypothalamic sites. Within the present study, this hypothesis will be tested by investigating the efferent connections that are involved in hypothalamically induced grooming behaviour and by comparing these efferents with projections of surrounding hypothalamic areas, where grooming could not be elicited.

Grooming behaviour has been elicited by electrical stimulation of a rostral hypothalamic area that includes parts of the hypothalamic paraventricular nucleus (PVH) and adjacent dorsal hypothalamic area (DHA) [47]. Local injections with various substances have indicated that neuronal cell bodies are involved in the elicitation of this behaviour [42,46,80,81,100].

In this study, the hypothalamic "grooming area" (HGA) was delineated using the effective injection sites that have been reported previously [47,80,81,100]. The specificity of the efferent connections of the HGA was investigated by comparing these efferents with the efferent projections of hypothalamic "non-grooming" sites outside and adjacent to the HGA.

The efferent connections of the PVH have already been described extensively and repeatedly (see e.g. [54,96] for a review). However, the hypothalamic area where grooming behaviour can be elicited is not confined to the cytoarchitectonic boundaries of the PVH, but tends to be concentrated in an area including (parts of) the PVH and adjacent dorsal hypothalamic area (DHA) [47,80,81,100]. For a complete description of the efferent connections involved in hypothalamically induced grooming behaviour it is therefore necessary to study all parts of the HGA.

In the present study, the anterograde tracer *Phaseolus vulgaris* Leucoagglutinin (PHA-L) has been used. The advantage of this tracer is that it shows not only individually labelled neurons within the injection site and the final destination of the efferent fibres,

but also the morphological characteristics of fibres "en route" [26].

Our PHA-L results will be compared with the distribution of oxytocinergic fibres, as a peptidergic system, originating from the PVH, but also from neurons within the DHA [77,87,96]. In addition, oxytocin appears to be involved in the elicitation of grooming behaviour, since intracerebroventricular injections of oxytocin enhance novelty induced grooming and local injections of oxytocin into the lateral hypothalamic area and ventral tegmental area induce grooming behaviour [20,37,38,68,101].

In this study we will assess whether projections of the HGA terminate in other brain areas which are involved in grooming behaviour. Electrical stimulation of the periventricular hypothalamic area, medial preoptic area and anterior hypothalamic area elicits grooming in the opossum [36,78]. Electrical stimulation of the parabrachial area, the noradrenergic A5 region and the area of the nucleus fastigii elicits elements of grooming behaviour in the cat [5,6]. Electrical stimulation of the locus coeruleus elicits grooming behaviour in the rat [2,58]. Local injection of various substances in the ventral tegmental area [37,98], the mesencephalic central gray [23,90] and the substantia nigra [91] have been reported to elicit grooming behaviour. In a study on the brain sites, that are involved in bombesin induced anorexia, Kyrkouli et al. [46] reported an increase in grooming behaviour by bombesin injection into the lateral hypothalamic area, dorsomedial hypothalamic nucleus, ventromedial hypothalamic nucleus, anterior hypothalamic area and posterior hypothalamic area.

Materials and methods

Anterograde tracing. In our study, 46 Wistar rats (Central Animal Laboratory, Nijmegen, The Netherlands) were used. Under pentobarbital anaesthesia (Narcovet, Organon, 1 ml/kg) the animals were placed in a stereotaxic apparatus. Iontophoretic deliveries (3-5 μA, 10 min.) of *Phaseolus vulgaris* leucoagglutinin (Vector Laboratories, Burlingame, U.S.A.: 5% in 0.1 M sodiumphosphate buffer (PB), pH 7.6) were made and aimed at the coordinates AP 1.6 mm, ML 0.2 mm and DV 7.6 mm [67]. After a survival time of 7-14 days, the animals were deeply anaesthetized with pentobarbital (1.5 ml/kg) and transcardially perfused with 100 ml saline and subsequently 300 ml fixative (2.5 % paraformaldehyde, 0.05 % glutaraldehyde in 0.1 M PB saline, pH 7.6). Brains were taken out of the skull and stored overnight in 20 % sucrose solution (PB, pH 7.6). Transverse 40 μm freeze sections were cut and 2 out of 5 sections were stained for PHA-L detection, one of which was counterstained with Nissl staining. Sections were rinsed for 2 hrs with Tris buffered saline (TBS). Sections were incubated at room temperature overnight in TBS containing biotinilated anti-Phaseolus antibody (Vector Laboratories: dilution 1:2000 in TBS-0.5% Triton X-100). After rinsing with TBS (3x) sections were stained using the ABC method (Vectastain, Vector Laboratories) for 90 min and stained with diaminobenzidine (DAB, 50 mg/100 ml Tris-HCl, pH 7.6), using 600 mg/100 ml ammonium nickel sulphate for staining intensification. Sections were coverslipped with Entellan.

Fibre patterns were drawn in the atlas of Paxinos and Watson [67], of which the hypothalamic and preoptic regions were redrawn using the detailed atlas of Geeraedts at al. (1990a,b) [24,25]. Part of the terminology that is used in this report is derived from Geeraedts et al. (1990a,b) [24,25].

In addition, four series of PHA-L injections were cut sagittally in order to study specifically the characteristics of the fibres "en route".

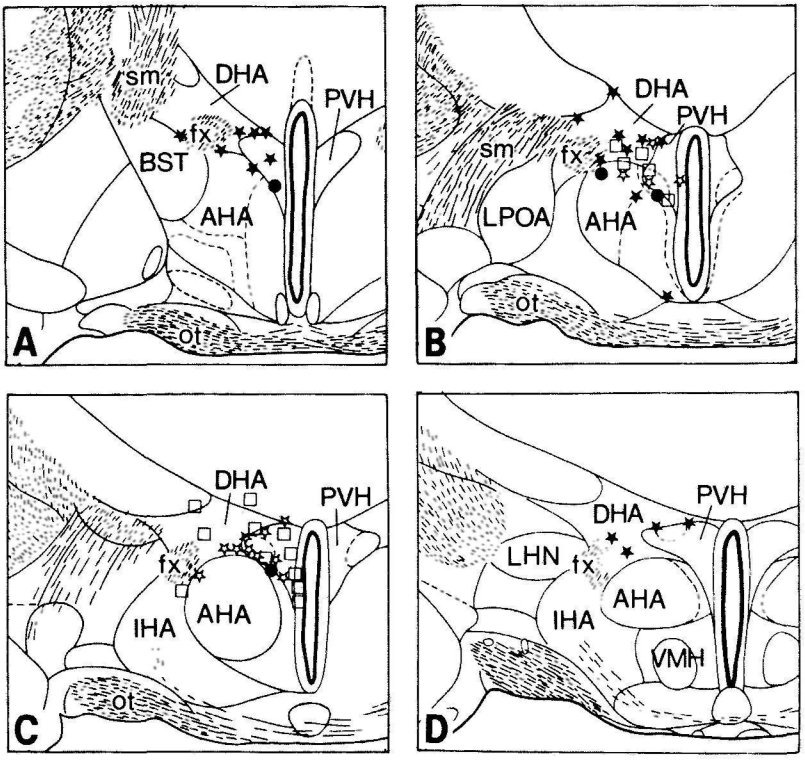


Figure 1 *Replotting of injection sites where grooming has been elicited in the hypothalamus. ✶: Roeling et al., 1990; ✰: Van Erp et al., 1991; □: Lammers et al., 1987; ●: Roeling et al., 1991. Drawings from rostral (A) to caudal (D) from the atlas of Geeraedts et al.* [24,25]. *For abbreviations: see list of abbreviations.*

Oxytocin staining. Four rats were perfused with Somogyi fixative (4% paraformaldehyde, 0.05% glutaraldehyde, 0.05% picric acid in 0.1 M PB, pH 7.4). After 2 hrs. of postfixation, brains were removed from the skull and rinsed overnight in TBS. Vibratome sections with a thickness of 75 µm. were cut. Two brains were sectioned transversally and two brains sagittally. Sections were rinsed in TBS and preincubated with incubation fluid (TBS, 0.5% Triton X-100, 0.1% bovine serum albumin (BSA), 5% Normal Swine Serum) for 1 hr. Incubation with anti-oxytocin (polyclonal, IncStar, dilution 1:4000 in preincubation fluid) occurred for 16 hrs at room temperature. After rinsing, sections were incubated with peroxidase-conjugated Swine- anti- Rabbit antiserum (1:100) for 2 hrs. Sections were stained with DAB (20 mg/ 100ml Tris buffer, 0.05 M, pH 7.6). Staining intensification with ammoniumnickelsulphate (600 mg/100 ml Tris buffer, 0.05 M, pH 7.6) was used. 10 µl H_2O_2 (30%) was added to start the staining reaction.

List of abbreviations.

7n	facial nerve
A5	noradrenergic A5 area
AA	amygdaloid area
ac	anterior commissure
Acb	nucleus accumbens
ACo	anterior cortical nucleus
AHA	anterior hypothalamic area
AHiA	amygdalohippocampal area
Arc	hypothalamic arcuate nucleus
Bar	Barrington's nucleus
BLA	basolateral amygdaloid nucleus, anterior
BLP	basolateral amygdaloid nucleus posterior
BMA	basomedial amygdaloid nucleus anterior
BMP	basomedial amygdaloid nucleus posterior
BST	bed nucleus stria terminalis
BSTIA	bed nucleus stria terminalis intrahippocampalis
C1/A1	medullary C1/A1 area
CA1	hippocampal CA1 area
CeA	central amygdaloid nucleus
CG	mesencephalic central gray
CGPn	central gray, pons
CI	colliculus inferior
Cl	claustrum
CM	centromedial thalamic nucleus
CnF	cuneiform nucleus
cp	cerebral peduncle
CPu	caudatoputamen
CS	colliculus superior
CTF	central tegmental field
dac	decussation anterior commissure
DBB	diagonal band Broca
DEn	dorsal entorhinal nucleus
DMH	dorsomedial hypothalamic nucleus
DMV	dorsal motor nucleus vagal nerve
DPM	dorsal premammillary nucleus
DR	dorsal raphe nucleus
fr	fasciculus retroflexus
fx	fornix
GP	globus pallidus
ic	internal capsule
IHA	intermediate hypothalamic area
I aA	lateral amygdaloid nucleus
LC	locus coeruleus
LHA	lateral hypothalamic area
LHb	lateral habenular nucleus
LHVL	lateral hypothalamic nucleus, pars ventrolateralis
LPB	lateral parabrachial nucleus
LPOA	lateral preoptic area
l Rt	lateral reticular nucleus
LSD	lateral septal nucleus, dorsal part
LSI	lateral septal nucleus intermediate part
LSO	lateral superior olive nucleus
LSV	lateral septal nucleus ventral part
MD	mediodorsal thalamic nucleus
MdD	dorsal medullary reticular field
MdV	ventral medullary reticular field
ME	median eminence
MeA	medial amygdaloid nucleus
MG	medial geniculate nucleus
ml	medial lemniscus
mlf	medial longitudinal fasciculus
mt	mammillothalamic tract
MnPO	median preoptic nucleus
MnR	median raphe nucleus
MPB	medial parabrachial nucleus
MPOA	medial preoptic area
MS	medial septal nucleus
NTS	nucleus solitary tract
ot	optic tract
OT	nucleus optic tract
OTU	olfactory tubercle
ox	optic chiasma
OVLT	organum vasculosum of the lamina terminalis
PeAV	anterior periventricicular nucleus
PHA	posterior hypothalamic area
PIl	posterior intralaminar thalamic nucleus
Pir	piriform cortex
PMCo	cortical amygdaloid nucleus posteromedial part
PMR	perimedian raphe nucleus
POM	medial preoptic nucleus
POMA	magnocellular preoptic nucleus
PP	peripeduncular nucleus
PPTg	nucleus tegmentalis pedunculopontinus
PrC	precommissural nucleus
PVH	paraventricular hypothalamic nucleus
PVT	paraventricular thalamic nucleus
py	pyramidal tract
Re	thalamic nucleus reuniens
Rh	thalamic rhomboid nucleus
RMg	nucleus raphe magnus
RPO	rostral periolivary region
rs	rubrospinal tract
Rt	nucleus rotundus thalami
SCh	suprachiasmatic nucleus
scp	superior cerebellar peduncle
SIC	substantia innominata, pars subcommissuralis
SIL	substantia innominata, pars sublenticularis
sm	stria medullaris
SNC	substantia nigra, pars compacta
SNR	substantia nigra, pars reticulata
SON	supraoptic nucleus
st	stria terminalis
SUT	subthalamic nucleus
TUM	medial tuberal nucleus
VL	ventrolateral thalamic nucleus
VLM	ventrolateral medulla
VLTg	ventrolateral tegmental area
VMH	ventromedial hypothalamic nucleus
VPM	ventral premammillary nucleus
VTA	ventral tegmental area
VTg	ventral tegmental nucleus
ZI	zona incerta

Results

Delineation of the hypothalamic "grooming" area.

In figure 1, the hypothalamic sites have been indicated where grooming has been elicited by electrical stimulation [47], or by local injection of kainic acid [80], N-methyl-D-aspartate (NMDA) [81], or adrenocorticotropic hormone (ACTH) [100]. The largest number of positive injection sites is found around the magnocellular part of the paraventricular nucleus (PVH), but positive injection sites are found throughout the dorsal hypothalamic area (DHA). A large amount of injection sites is situated in the small zone of the anterior hypothalamic area ventral to the paraventricular nucleus. The delineation of the area, that is indicated in the present report as hypothalamic "grooming" area (HGA) is shown in figure 2 by small stippling.

"Grooming area" injections, control injections and oxytocinergic projections.

In Fig. 9, an example is presented of an injection site within the HGA (case R146). Labelled neurons with first and sometimes second order dendrites can be distinguished. From the injection site, labelled, mainly thin, fibres spread in various directions. Many of these fibres contain varicosities along their course rostrally or caudally. Since these varicosities may contain synapses [106], we decided to describe not only the destination but also the course of these efferent fibre streams.

From the total series of 46, twelve injection sites have been selected for further study (Fig. 2), seven of which were placed inside the HGA. Two HGA injection sites were strictly confined to the borders of the PVH (R170, 171). Two HGA injection sites included both PVH and DHA (R111, R172), two injection sites were situated in the DHA (R112, R146) and one site had its largest extent in the small zone ventral to the PVH (R156). Labelled fibres from a DHA injection are shown in figure 5 (Rat R146). Labelled neurons in this case are almost exclusively found in the DHA, with only a few labeled neurons in the dorsal part of the anterior hypothalamic area, adjacent to the DHA, and in the zona incerta. Efferents from a PVH injection are shown in figure 6 (Rat R170). The small injection in this case is strictly confined to the parvocellular part of the PVH (Fig.10).

For comparison, efferents of five PHA-L injection sites around the HGA have been analysed (R207, R156, R140, R142, R293). Fig. 8 illustrates the efferent fibres of an injection that is placed ventral to the HGA, within the dorsal aspects of the anterior and intermediate hypothalamic areas (rat R207).

The origin and course of the oxytocinergic fibres are shown in figure 7. Within the rostral hypothalamus, oxytocinergic neurons are located in the paraventricular nucleus (PVH) and supraoptic nucleus (SO), but also in the lateral aspect of the dorsal hypothalamic area (DHA), in between the fibres running towards the pituitary, in the dorsal part of the medial preoptic area (MPOA), and in the pars sublenticularis of the substantia innominata (SIL) (Fig.7C, Fig.14).

In Table 1, a semiquantitative listing is presented of the amount of labeled fibres in a number of brain areas.

In the description of the efferent connections, only the ipsilateral side of the brain

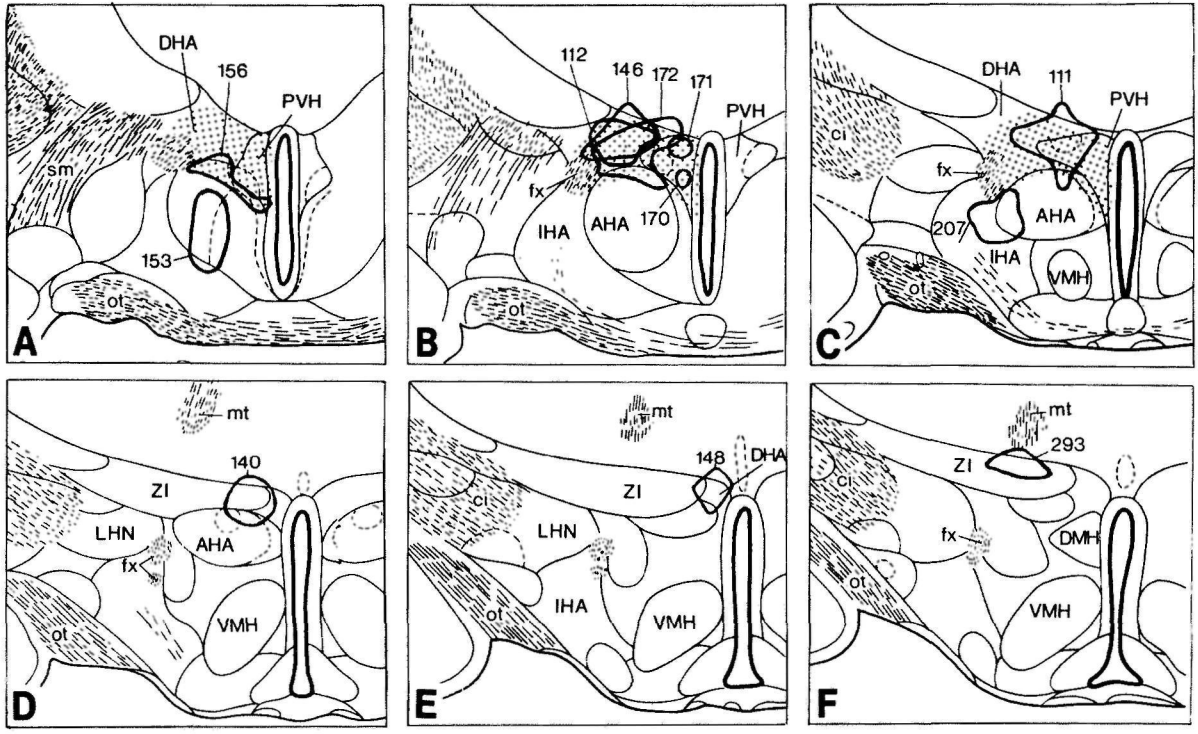


Figure 2 *Injection sites that are used in the present study. The largest extension of the injection sites are plotted in the atlas of Geeraedts et al.[24,25]. The hypothalamic "grooming area" is indicated with stippling. For abbreviations: see list of abbreviations.*

will be described. In every experimental series, fibres in contralateral brain areas have also been identified, but always to a much lesser extent as compared to the ipsilateral side. In the topography of the fibres on both sides no marked differences have been found.

Efferent fibre patterns from the HGA and control sites.

The general pattern of efferent fibres within the hypothalamus is very diffuse. However, by tracing the fibres in different directions, a number of fibre "streams" can be distinguished. In figure 3, "flow patterns" are indicated for the fibres leaving the dorsal hypothalamic area (3B, rat R146), the paraventricular nucleus (3C, rat R170) and the intermediate/ anterior hypothalamic area (3D, rat R207). Figure 4 shows the efferent fibre "streams" from the DHA injection in more detail. To rostral, three ascending fibre "streams" have been distinghuished: a dorsal, a ventromedial and a lateral ascending fibre stream. To caudal, three descending fibre streams have been distinghuished: a dorsal, a ventral and a lateral fibre stream. Two thalamic fibre streams will be described as well: a dorsal and a ventral thalamic fibre stream.

The dorsal ascending fibre stream

Fibres within the dorsal ascending fibre stream (stream 1, Fig.3, 4) innervate the **bed nucleus of the stria terminalis** (BST) and partially terminates here with varicosities and terminal arborizations. The bed nucleus of the stria terminalis receives large efferent projections from all hypothalamic injection sites that were studied (Figs. 5B, 6B, 7B, 8B). From this stream, fibres enter the stria terminalis. These fibres are smooth with occasional varicosities. Interestingly, no PVH fibres are found entering the stria terminalis (Fig.3C, 6B) and only sparse oxytocinergic fibres (Fig.7B).

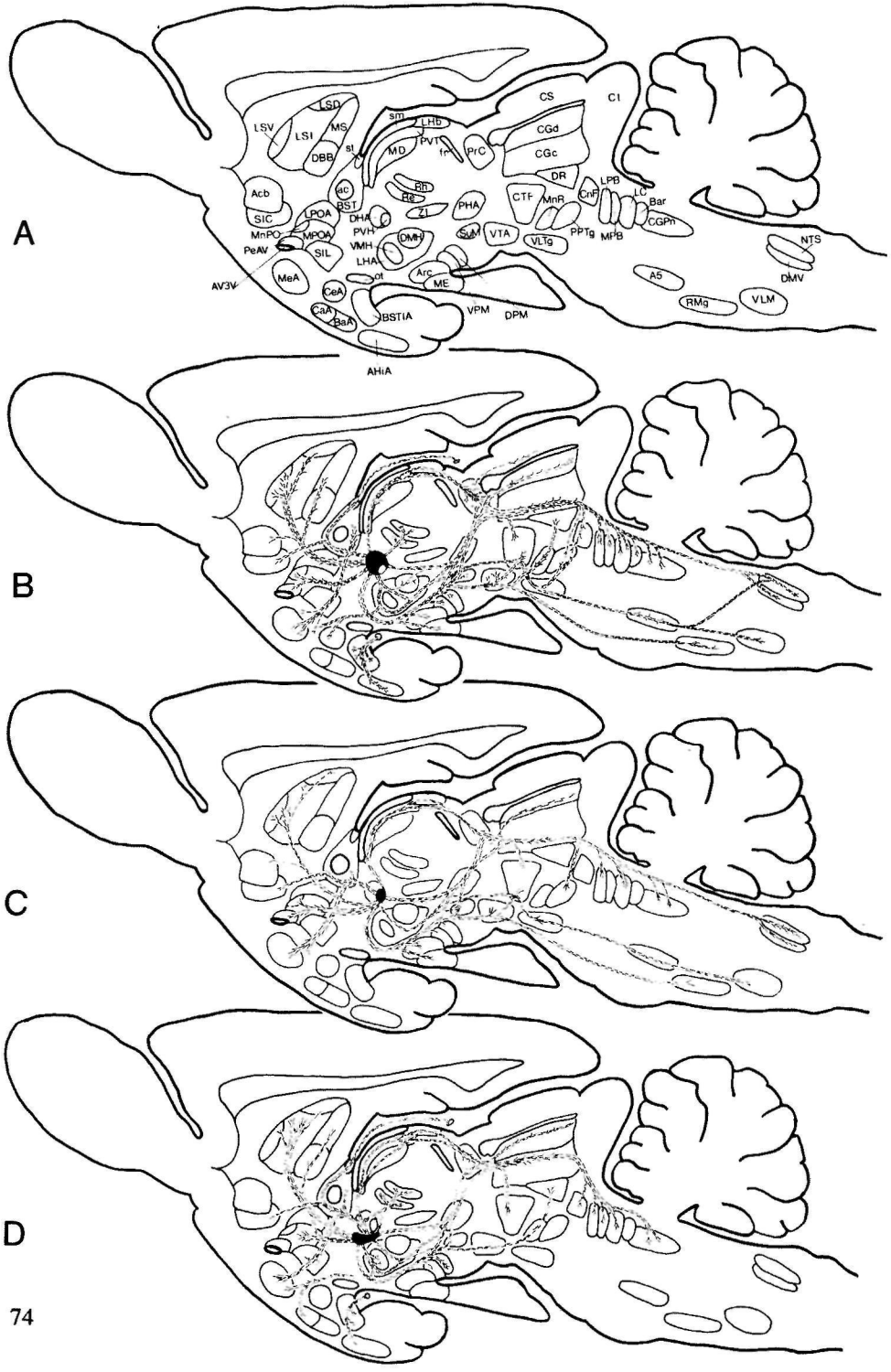
Within the BST the stream diverts and enters the **substantia innominata, pars subcommissuralis** (SIC) or the **substantia innominata, pars sublenticularis** (SIL) (Fig.3).

A moderate number of fibres from the hypothalamic "grooming area" (HGA) are found in the SIC. This projection appears to be larger than projections from non-HGA sites, except for a zona incerta injection (R293) (Figs. 5B, 6B, 8B, table 1). Within the SIL, a moderate number of fibres from all injection sites is present (Figs. 5B-C, 6B-C, 7B-C, 8B-C, table 1). A moderate number of oxytocinergic fibres is found in the SIC and in the SIL (Fig.7A-C).

A number of fibres runs through the SIL and enter the amygdala (Fig.3). The **anterior amygdaloid area** (AA) and **medial amygdaloid nucleus** (MeA) are moderately innervated by DHA- and oxytocinergic fibres (Figs.5B-D, 7B-D). PVH fibres innervate the AA and MeA as well, but the number of these fibres is considerably smaller (Figs.6B-D). Control injections in the IHA and AHA project moderately to the AA and MeA (Figs.8B-

next page:

Figure 3 *Schematic sagittal "flow charts" of efferent fibre streams after injections in the dorsal hypothalamic area (3B), paraventricular hypothalamic nucleus (3C) and intermediate/ anterior hypothalamic area (3D). For abbreviations used in 3A: see list of abbreviations.*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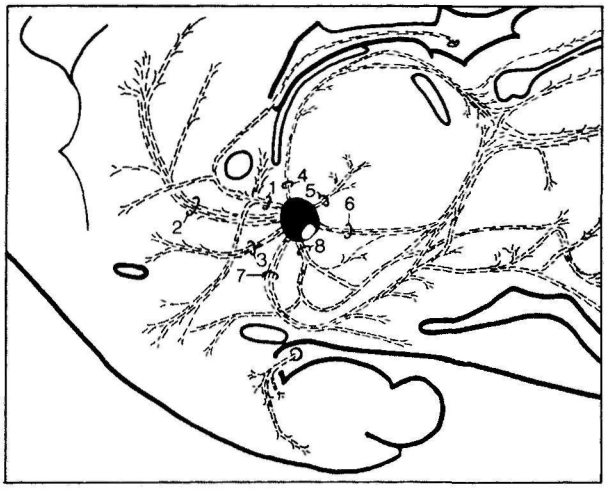


Figure 4 *Detail of figure 3B. Schematic indication of efferent fibre streams after a PHA-L injection into the dorsal hypothalamic area.*

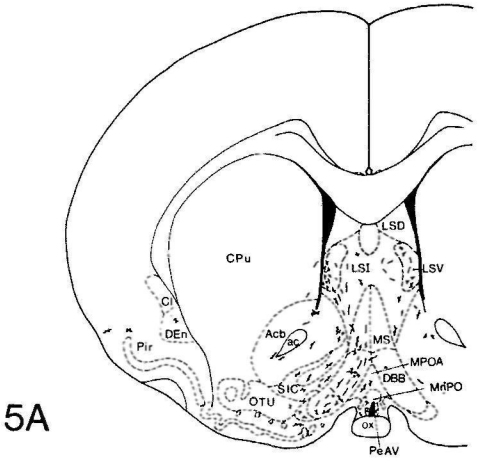
D), except for the small injections in cases R140 and R148 (zona incerta and caudal part of the dorsal hypothalamic area, respectively). The **central amygdaloid nucleus** (CeA) is innervated by DHA fibres and oxytocinergic fibres, but not by fibres from the PVH injection sites. From the control injections, only an injection in the IHA/ AHA area (R207), no labelled fibres in the CeA were found after control injections. At caudal levels, the **intra-amygdaloid part of the bed nucleus of the stria terminalis** (BSTIA) is innervated by fibres from the DHA, IHA and AHA and by oxytocinergic fibres (Figs.5E, 8E, 7E). A part of the fibres in the BSTIA extend to the **amygdalohippocampal area** (AHiA). The PVH and zona incerta do not appear to project to the BSTIA and AHiA.

The lateral ascending fibre stream.

The lateral ascending fibre stream (stream 2, Fig.3, 4) ascends via the lateral preoptic area (LPOA) towards the lateral septal nucleus through the transition between the lateral preoptic area (LPOA) and lateral septal nucleus. Within the LPOA, these fibres are

next page:

Figures 5,6,7,8 *Drawings of efferent projections of a DHA injection (Fig.5, R146), a PVH injection (Fig.6, R170), Oxytocinergic neurons (Fig.7) and a AHA/ IHA injection (Fig.8, R207). The δδ in the septum, that are shown in figures 8A and 8B represent pericellular baskets (see text). For abbreviations: see list of abbreviations.*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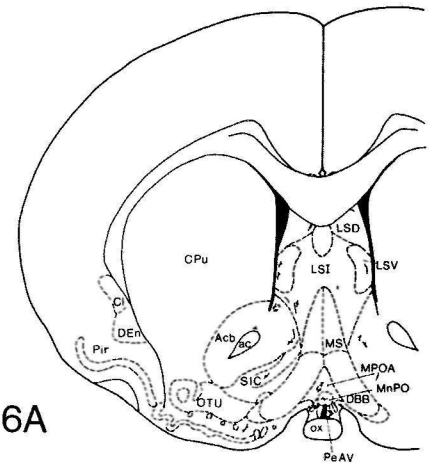
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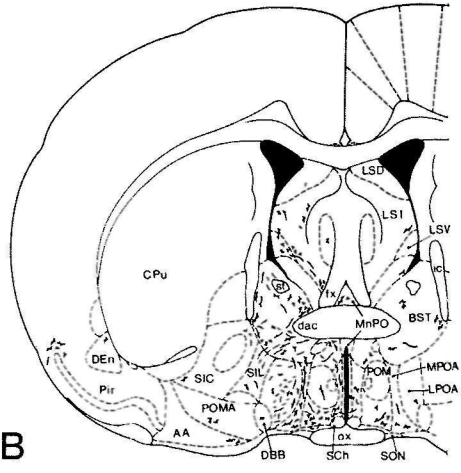
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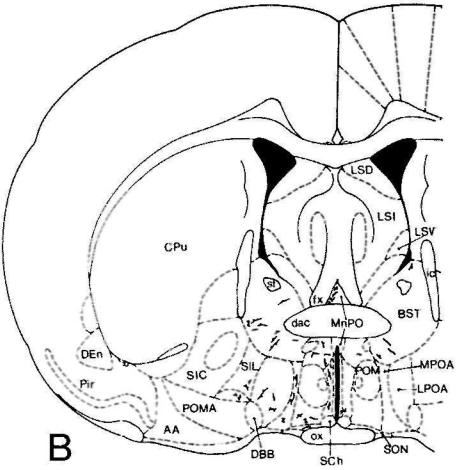
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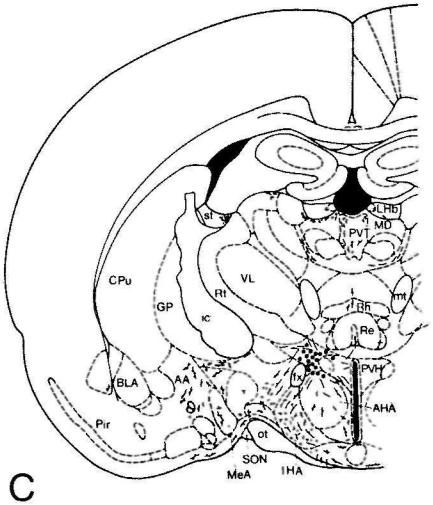
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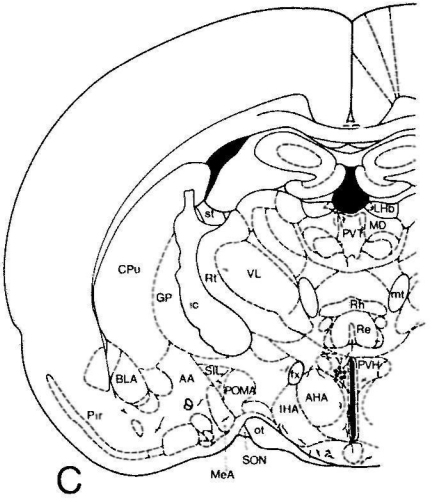
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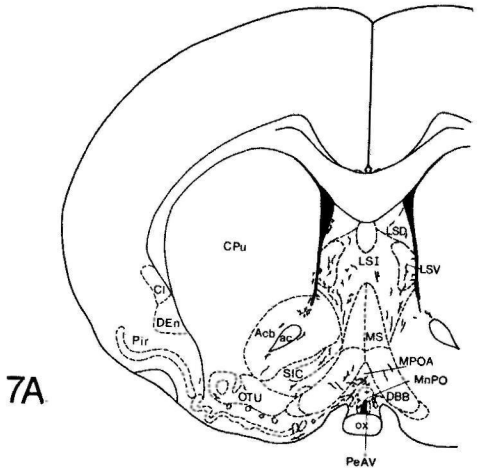
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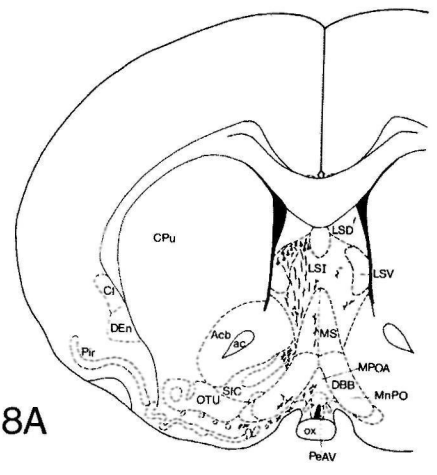
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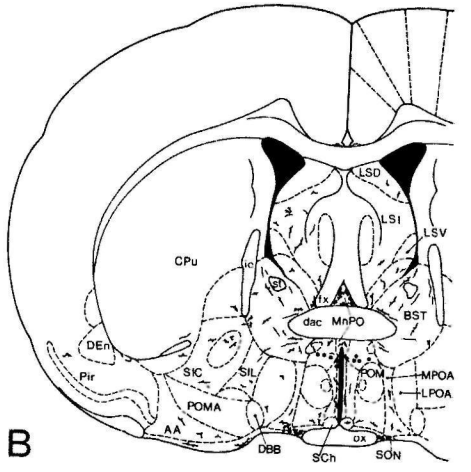
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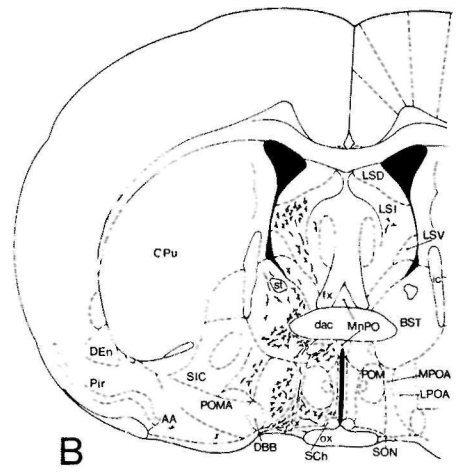
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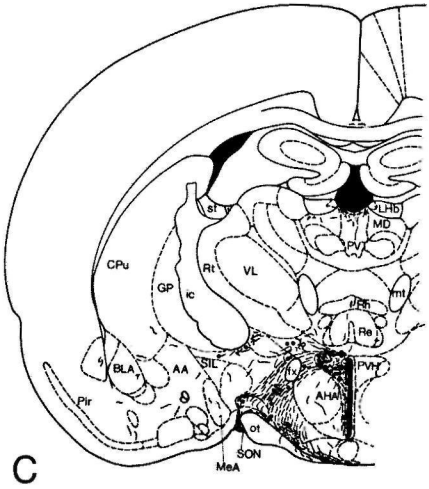
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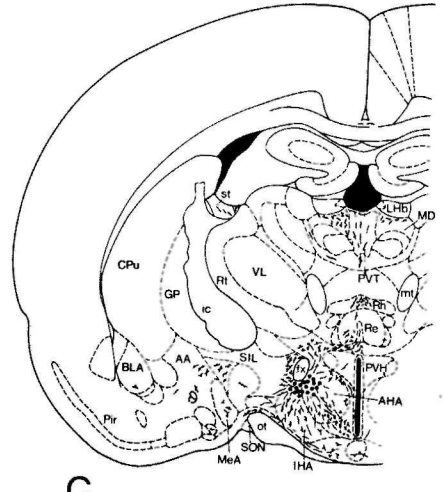
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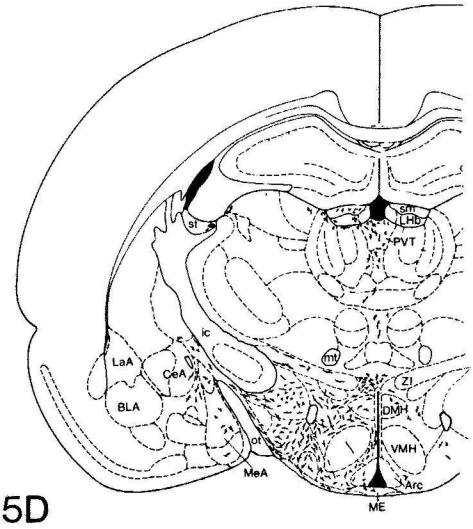
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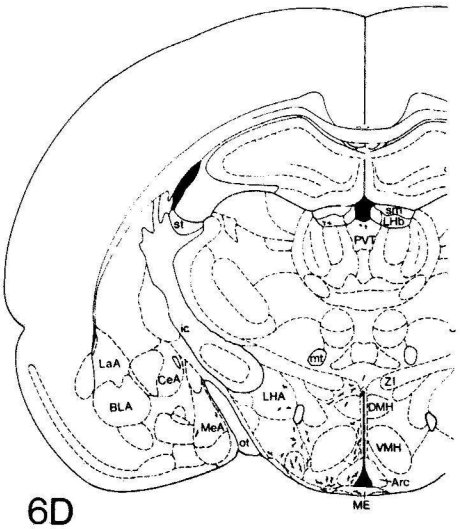
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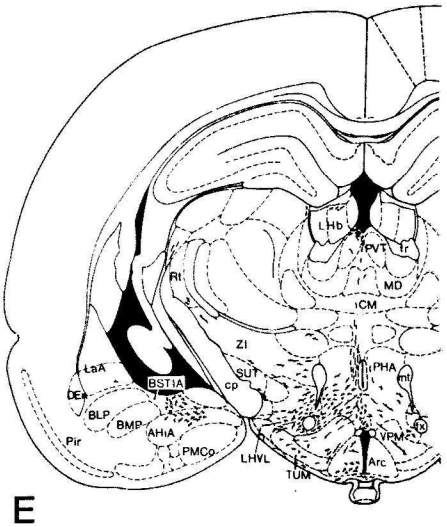
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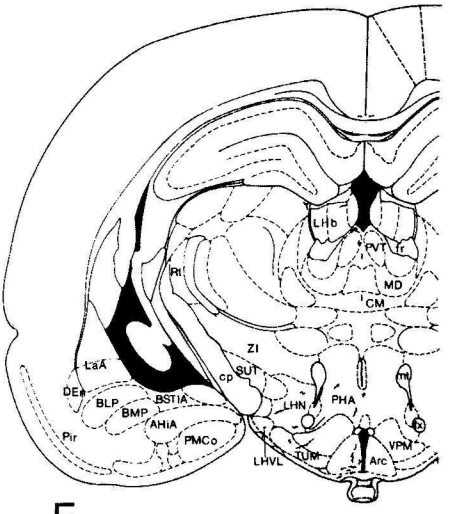
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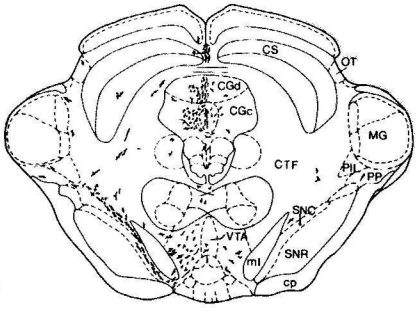
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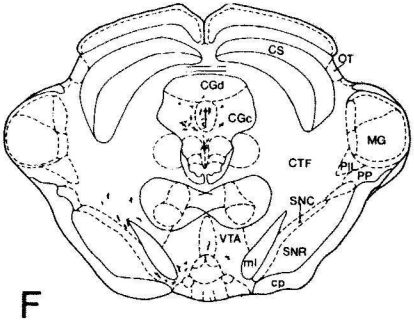
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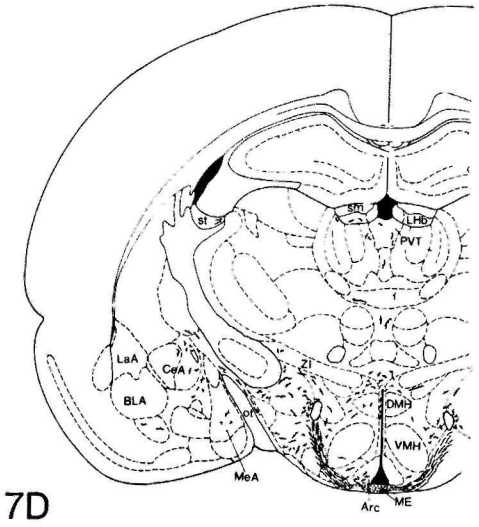
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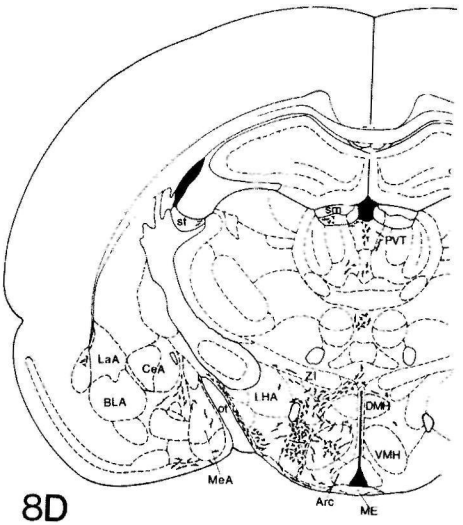
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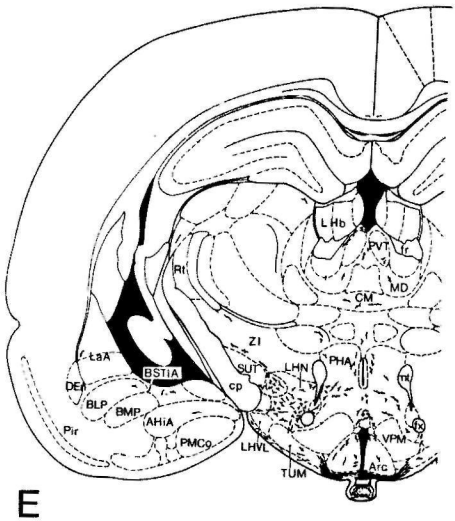
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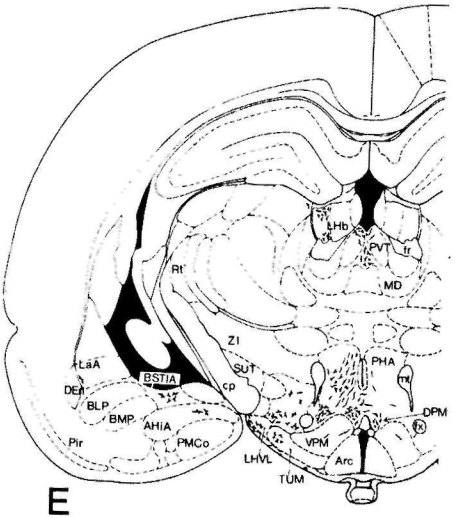
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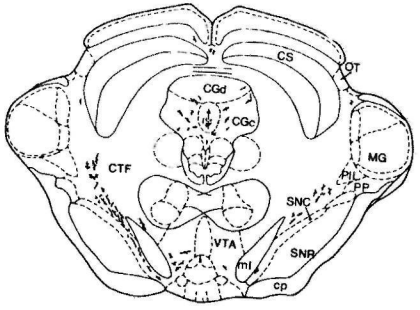
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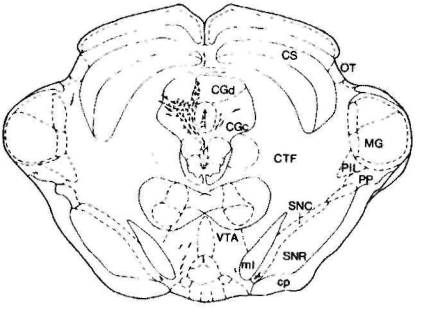
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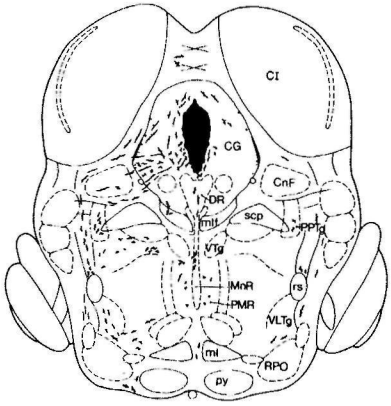
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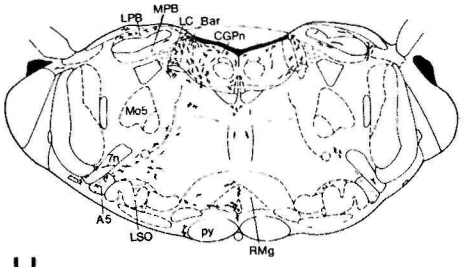
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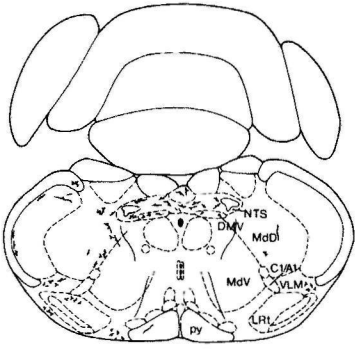
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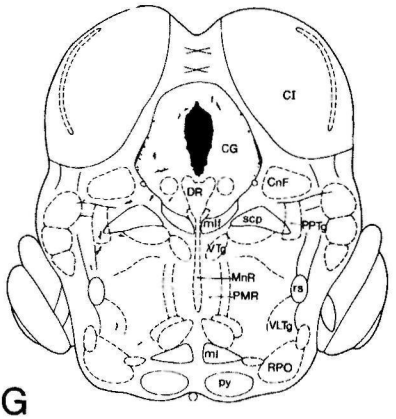
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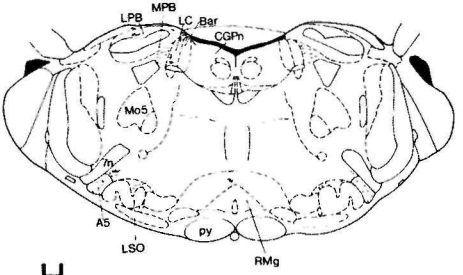
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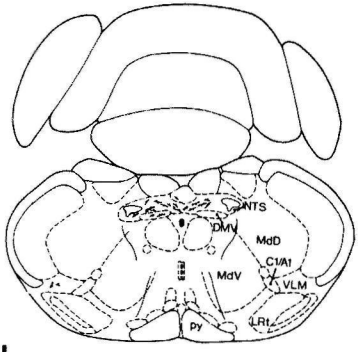
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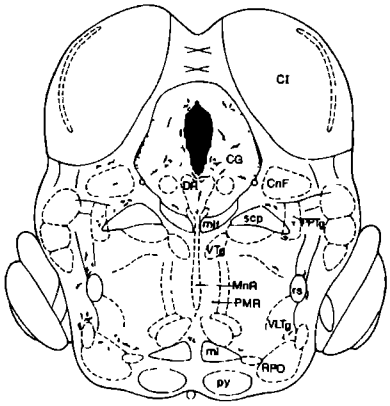
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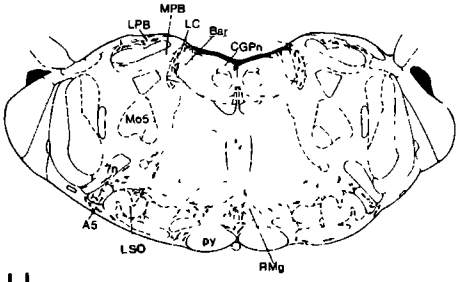
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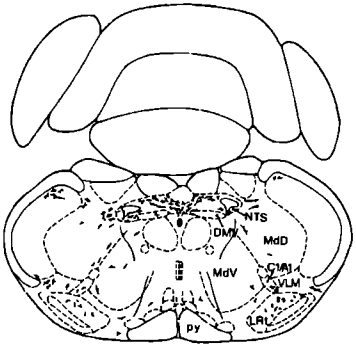
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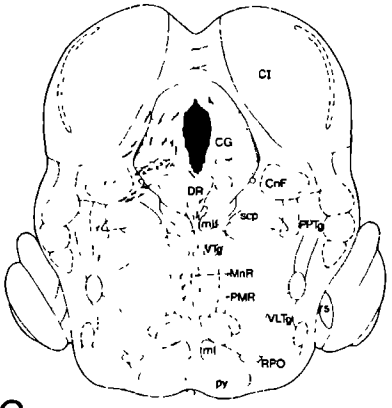
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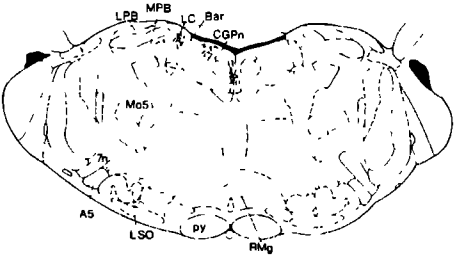
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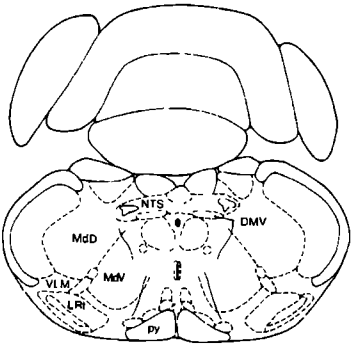
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mainly smooth, with few varicosities. Within the septal nucleus, the number of varicosities increases. Other fibres from this stream enter the diagonal band and medial septal nucleus.

The **lateral preoptic area** (LPOA) is heavily innervated by fibres from the dorsal hypothalamic area (Fig.5B), but only sparsely by fibres from the PVH (Fig. 6B) and oxytocinergic fibres (Fig.7B). The number of fibres form hypothalamic sites outside the hypothalamic "grooming area" (HGA) projecting to the LPOA vary in number (Fig.8B, Table 1).

The **septal** innervation appears to have a spatial organisation. Within the septum, varicose fibres and terminal arborizations from the DHA concentrate in the **ventral part of the lateral septum** (LSV), directly apposed to the wall of the lateral ventricle (fig. 3A, 11). Some fibres appear to be positioned between the cells of the ependymal layer and are running perpendicular to the wall of the ventricle. A sparse number of varicosities were found on these fibres. Only sparse fibres and no terminals are found in the **dorsal part of the lateral septum** (LSD). A few varicose fibres originating in the dorsal hypothalamic area innervate the **medial septal nucleus** (MS) in cases R146 (fig. 5A) and R172, but are absent in two other dosral hypothalamic area injections (cases R111 and R112). Only a few varicose fibres are found in the diagonal band of Broca (Fig.5A).

Septal innervation by PVH neurons is only sparse. Like fibres from the DHA, PVH fibres are located in the ventral part of the lateral septal nucleus (LSV) and in the ventral aspects of the intermediate part of the lateral septum (LSI) (Fig.6A). Only sporadic fibres are present in the dorsal part of the lateral septal nucleus (LSD). No fibres are found in the **diagonal band** (DBB) and **medial septal nucleus** (MS) (Fig.6A). Oxytocinergic fibres display the same pattern as fibres originating in the dorsal hypothalamic area (Fig.7A). Most oxytocinergic fibres in the lateral septal nucleus are varicose. Some fibres enter the ependymal layer of the lateral ventricle. Only a few oxytocinergic fibres are found within the DBB, either running towards the medial septal nucleus or traversing the DBB (Fig 7A).

PHA-L labelled fibres from the IHA/ AHA injection (rat R207) appear to surround septal neurons in a specific way (Fig.8A, 12) These "pericellular baskets" are positioned in the intermediate part of the lateral septal area (LSI) apposed to the dorsal part of the lateral septal nucleus (LSD) (Fig.12). These baskets are not found in case R153 (AHA injection), in which case labelled fibres are found only in the ventral part of the LSI and only few fibres in the lateral dorsal aspects of the LSI. Within the LSD no labelled fibres are present. Other septal areas are only moderately innervated in case R207 (fig. 8A). Fibres in the ventral parts of the LSI and LSV are mainly smooth with a small number of varicosities. The DBB contains only few labelled fibres, that run towards the medial septal nucleus (MS).

Efferents from the zona incerta (R140) innervate the septum and terminals appear to cluster more dorsally than is described in case R146 (DHA). In case R293 (zona incerta, caudal to injection site R140), only sporadic fibres are found in the septum.

The ventromedial ascending fibre stream.

The ventromedial ascending fibre stream (stream 2, Fig.3, 4) ascends via the medial preoptic area, the area ventral to the preoptic nucleus, and the periventricular hypothalamic nucleus. Fibres in this stream have many varicosities all along their course rostrally. Within the preoptic area, fibres innervate the median preoptic nucleus (MnPO).

The medial ascending fibre stream is less pronounced in the non-grooming series than in the HGA series.

The **medial preoptic area** (MPOA) is innervated by many varicose fibres from DHA and PVH. A moderate number of oxytocinergic fibres innervates this area as well. Many fibres are found in the ventral part of the medial area, ventral to the medial preoptic nucleus (POM), which remains virtually empty itself (Figs.5-7B). The medial preoptic area (MPOA) is also innervated by other parts of the hypothalamus outside the HGA (Fig.8B). Injections made in the zona incerta reveal no labelled fibres in the MPOA (Table 1).

The **median preoptic nucleus** (MnPO) is well innervated by varicose fibres from all injection sites within the HGA (Figs. 5A-B, 6A-B, Table 1), as well as by oxytocinergic fibres (fig. 7A-B). In contrast, no labelled fibres from injection sites outside the HGA are found in this area (Fig. 8A-B, table 1).

Fibres from the ventromedial ascending fibre stream can be followed entering the anteroventral parts of the periventricular nucleus (PeAV) and rostral parts of the medial preoptic area (MPOA). Some fibres from this stream join hypothalamic fibres running within the lateral ascending fibre stream.

Fibres from injection sites within the HGA, as well as oxytocinergic fibres, are found in between the cells of the ependymal layer of the anteroventral recess of the third ventricle near the organum vasculosum of the lamina terminalis (OVLT) in the **anteroventral periventricular region** (PeAV) (Fig 5A, 6A, 7A). These fibres have many varicosities in this area.

Neurons from the anterior hypothalamic area, intermediate hypothalamic area and zona incerta do not innervate this region (Fig.8A, table 1). In case R148 (caudal part of the DHA), a moderate number of labelled fibres was found in the PeAV (Table 1).

Dorsal thalamic fibre stream.

Fibres within the dorsal thalamic stream (stream 4, Fig.3, 4) enter the thalamus rostrally, caudal to the decussation of the anterior commissure (dac) and medial to the stria medullaris (sm). The fibres turn dorsally and caudally, staying in the dorsal part of the midthalamic region, within parts of the thalamic paraventricular nucleus (PVT), parts of the mediodorsal thalamic nucleus (MD), in a small area ventral to the stria medullaris (sm), within the sm itself, and within the lateral habenular nucleus (LHb), until they reach the mesencephalon. The fibres have many varicosities all along their way caudally. A large number of fibres are positioned perpendicular to the dorsal edge of the PVT.

The general pattern of thalamic innervation was identical in all series. In the **thalamic paraventricular nucleus** (PVT) and **lateral habenular nucleus** (LHb) a moderate number of labelled fibres was found in all series examined (Figs 5D-F, 6D-F, 7D-F, 8D-F, Table 1). Most injection sites within the HGA resulted in a small number of labelled fibres in the **mediodorsal thalamic nucleus** (MD), but hypothalamic sites outside the HGA project to the MD as well. A particularly large innervation of the MD is found after PHA-L injection into the intermediate and anterior hypothalamic area (R207). Some labelled fibres run through the stria medullaris (sm) (Fig.5D, 8D), but no consistent pattern could be found when different injection sites were compared. The PVH does not appear to project to the **central thalamic nucleus** (Fig.6C-E), while a large number of fibres from the zona incerta is situated in the **lateral part of the central thalamic nucleus** (CL). Other injection sites resulted in only inconspicuous innervation of the central thalamic

nucleus (CL/ CM).

At the transition between diencephalon and mesencehalon, the fibres of the dorsal thalamic stream enter the mesencephalic central gray and cross the fibres that descended directly via caudal hypothalamic regions (see below). In this area of crossing and intermingling of fibres, many varicosities and terminals are found, particularly in the **precommissural nucleus** (PrC) (Fig.13).

The ventral thalamic fibre stream.

The ventral thalamic fibre stream (stream 5, Fig.3, 4) is a small contingent of fibres entering the thalamus through the midline and innervating the ventral midline thalamic nuclei. The fibres are mainly smooth, displaying varicosities only in the **rhomboid nucleus** (Rh) and **reuniens nucleus** (Re).

Fibres from the DHA and PVH running through this stream are only moderate in number (Figs.5C-D, 6C-D). Oxytocinergic fibres innervate the Rh and Re as well (Fig.7C-D). Small numbers of varicose fibres from non-HGA areas are also found in these nuclei (Fig.8C-D, Table 1).

Intrahypothalamic fibres.

In the hypothalamus, varicose fibres originating in the DHA can be seen at all levels in the lateral, intermediate and medial hypothalamic areas. Descending PVH fibres are mainly found in medial hypothalamic areas, around the ventromedial hypothalamic nucleus (VMH). Only a few descending varicose PVH fibres are positioned in the lateral hypothalamic area.

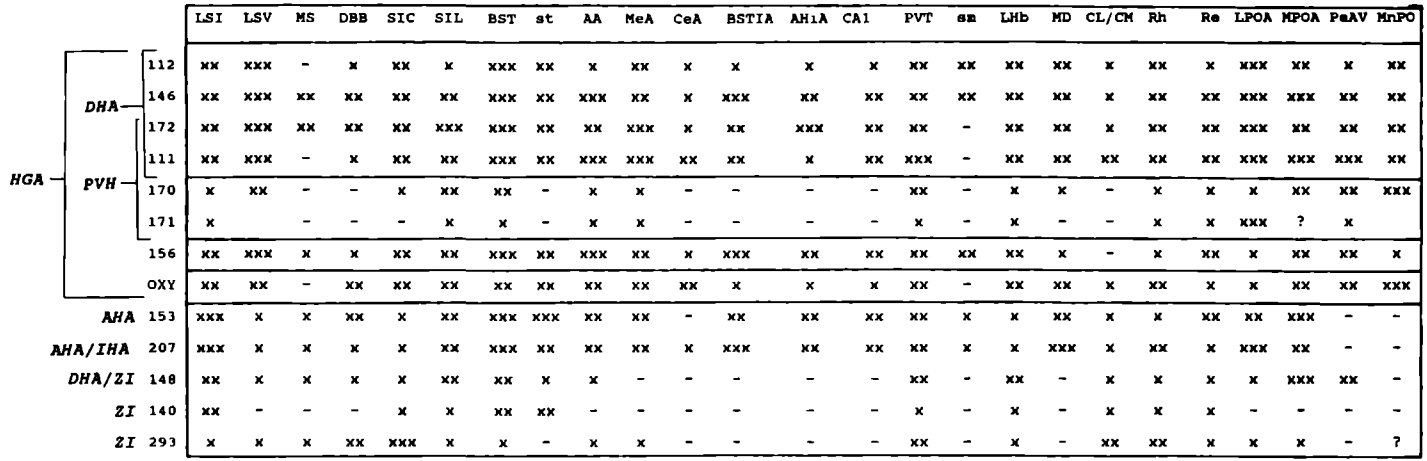
Oxytocinergic fibres are found throughout the entire caudal hypothalamus (Fig.6D). Some fibres enter the ventral part of the zona incerta (ZI). Descending fibres from the IHA/ AHA are mainly located in a small zone ventral to the fornix (Fig.8D). The lateral hypothalamic area is only sparsely innervated by fibres from this hypothalamic area.

The dorsal descending fibre stream.

The dorsomedial descending stream (stream 5, Fig.3, 4) is situated in the area medial and dorsal to the dorsomedial hypothalamic nucleus (DMH), running caudally (Fig.3, 4). The fibres are mainly varicose, intermingled with smooth fibres. This stream enters the mesencephalic central gray (CG) after traversing the posterior hypothalamic area (PHA). Within the PHA, mainly smooth fibres with few varicosities are found. At the transition zone between diencephalon and mesencephalon, fibres from this stream join the fibres from the thalamic tract (stream 4) and enter the central gray. Many varicosities and

next page:

Table 1 *Semiquantitative indication of innervation of various brain sites after PHA-L injections into the hypothalamic "grooming area" and surrounding hypothalamic regions. - no innervation; * sparse innervation; ** moderate innervation; *** strong innervation. For abbreviations: see list of abbreviations.*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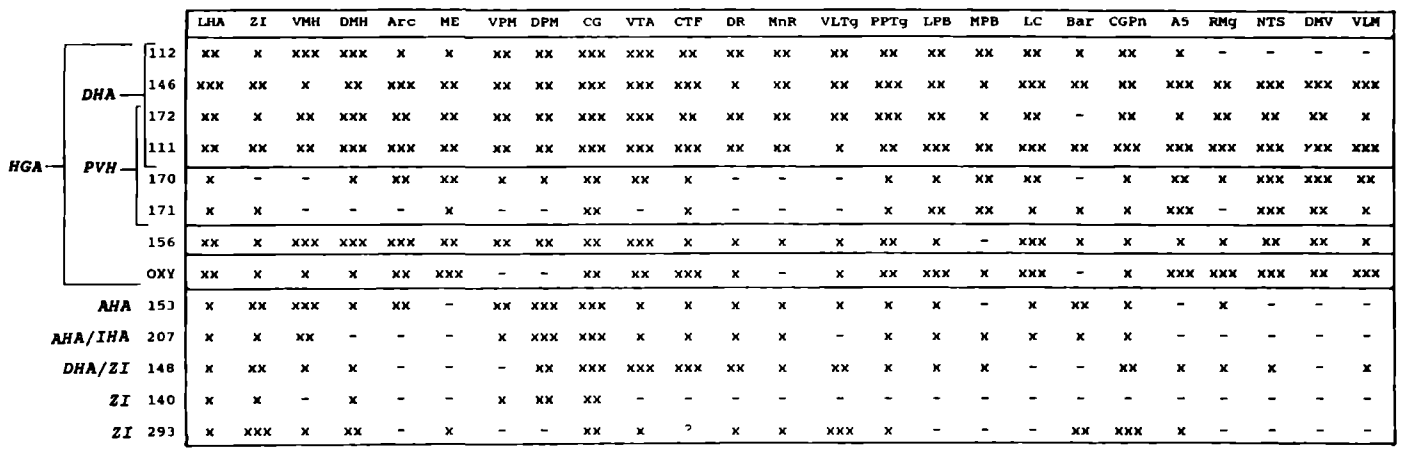
AHA 153
AHA/IHA 207
DHA/ZI 148
ZI 140
ZI 293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112
146
172
111
170
171
156
OXY
AHA 153
AHA/IHA 207
DHA/ZI 148
ZI 140
ZI 293

terminal arborizations can be found in the precommissural nucleus (PrC) (Fig.13).

The ventral descending fibre stream.

The ventral descending fibre stream (stream 6, Fig.3, 4) is situated in the intermediate hypothalamic area (IHA) and ventral to the ventromedial hypothalamic nucleus (VMH). Within this stream, smooth and varicose fibres are intermingled. Fibres from this stream innervate the arcuate nucleus (Arc) and the median eminence (ME). Some fibres turn dorsally and join the fibres of the dorsomedial descending stream, partially innervating the dorsomedial hypothalamic nucleus (DMH).

The **ventromedial hypothalamic nucleus** (VMH) is remarkably devoid of DHA and PVH innervation (Fig. 5D, 6D) and is only sparsely innervated by oxytocinergic fibres (Fig.7D). In the IHA/ AHA injection (R207), the VMH is innervated by varicose fibres and terminals that cluster in the central part of the VMH (Fig.8D). In case R153 (AHA injection), the VMH is heavily innervated with the largest amount of fibres localized in the dorsomedial part of the VMH. Fibres from the zona incerta innervate the VMH only sparsely (Table 1).

The hypothalamic **arcuate nucleus** (Arc) is moderately innervated by varicose fibres from both PVH and DHA injection sites, except for case R171 (dorsal parvocellular PVH). Oxytocinergic fibres innervate the arcuate nucleus as well (Fig.7D). From injection sites outside the HGA, only in case R156 (AHA injection) labelled fibres are found in the arcuate nucleus.

Fibres from DHA and PVH enter the **median eminence** (ME) (Fig.5D-E, 6D-E). A large number of oxytocinergic fibres is found in this area as well (Fig.7D-E). In control series, only sparse innervation of the median eminence by fibres from the zona incerta (R293) is found.

The **dorsomedial hypothalamic nucleus** (DMH) is moderately innervated by varicose DHA fibres and terminal arborizations (Fig.5D). The DMH is innervated by a few varicose PVH fibres (Fig.6D) and oxytocinergic fibres (Fig.7D). The DMH does not (R207) or only sparsely (R153, R148, R140, R293) receive fibres from our control injections outside the HGA.

In the mammillary region, fibres from the DHA innervate the **ventral and dorsal premammillary nuclei** (VPM, DPM). Only the PVH injection, that was placed in the ventral parvocellular part of the PVH (rat R171) showed labelled fibres in the VPM and DPM. No oxytocinergic fibres have been found in these nuclei. In the control series, the DPM was in general more strongly innervated by labelled fibres than the VPM (Fig.8E, Table 1).

The lateral descending fibre stream.

The lateral descending fibre stream (stream 7, Fig.3, 4) is situated in the lateral hypothalamic area (LHA), within the medial forebrain bundle [63]. This stream is much more pronounced in the DHA series than in the PVH and our "non-grooming" series. Some fibres leave the lateral stream to enter the amygdala between the optic tract and the capsula interna. Further caudally, a number of fibres turns dorsally and joins the fibres of the dorsal descending stream towards the mesencephalic central gray (see above). Other

fibres stay in a lateral position and enter the ventral part of the central tegmental field (CTF), forming the lateral descending fibre stream of the medial forebrain bundle [31]. Within the lateral hypothalamic area, a moderate innervation by DHA- and oxytocinergic fibres is found. Only a sparse number of fibres from the PVH and from the control sites is found in the LHA (Figs.6D-E, 8D-E, Table 1).

Brainstem projections.

Within the brainstem, the descending fibre streams are found in three streams: the dorsal descending fibre stream is continued in the dorsal brainstem fibre stream, running through the mesencephalic central gray; the ventral descending fibre stream is continued in the ventromedial brainstem fibre stream within the medial descending fibre stream of the medial forebrain bundle [31]. The lateral descending fibre stream is continued in the ventrolateral brainstem fibre stream, within the lateral descending stream of the medial forebrain bundle [31].

Dorsal brainstem fibre stream.

At the transition zone between diencephalon and mesencephalon, DHA fibres are concentrated dorsally in the **central gray**. Fibres in this area are running vertically, sometimes crossing the posterior commissure and terminating in a small area between both colliculi superiores (Fig.3, 5F). The same pattern was found for oxytocinergic projections (Fig.7F) and for projections from control injections (Fig.3, 8F), but not for fibres from the parvocellular PVH (Fig.6F).

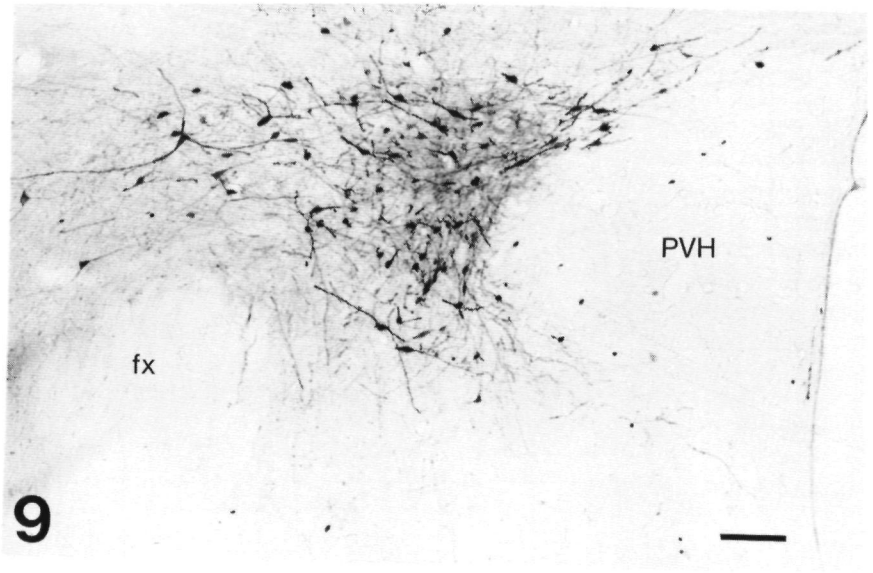
More caudally, DHA fibres within the CG are grouped in two clusters: one cluster is positioned in the dorsal aspects of the CG, the other in the ventrolateral aspects of the CG (Fig.5F). Both clusters are concentrated in the central parts of the CG, leaving the peripheral parts virtually empty. At more caudal levels, the dorsal cluster gradually disappears (fig. 5G).

PVH fibres and oxytocinergic fibres concentrate in the same rostrodorsal and caudoventral clusters in the central part of the CG, but the dorsal cluster was only small (Figs.6F-H, 7F-H). Some fibres in the periventricular part of the CG appear to be positioned within the ependymal layer and the ependymal ridges around the aqueduct. In case R156 (ventral rim of the PVH and DHA), labelled fibres cluster in the dorsal CG and dorsal part of the lateral CG. Further caudally, fibres are found in the ventrolateral CG. Fibres from the AHA/ IHA injection (rat R207) tend to cluster in the dorsal aspect of the lateral part of the CG, in both central and peripheral zones (Fig.8F).

Further caudally the number of labelled fibres in the CG in control series rapidly diminishes and no labelled fibres are found caudal to the pontine region, except for fibres

Figure 9 *Photomicrograph of the PHA-L injection site within the dorsal hypothalamic area (DHA) (case R146). Scale bar: 100 μm.*

Figure 10 *Photomicrograph of a PHA-L injection into the parvocellular part of the hypothalamic paraventricular nucleus (R170). The dashed line indicates the borders of the PVH. Scale bar: 100 μm.*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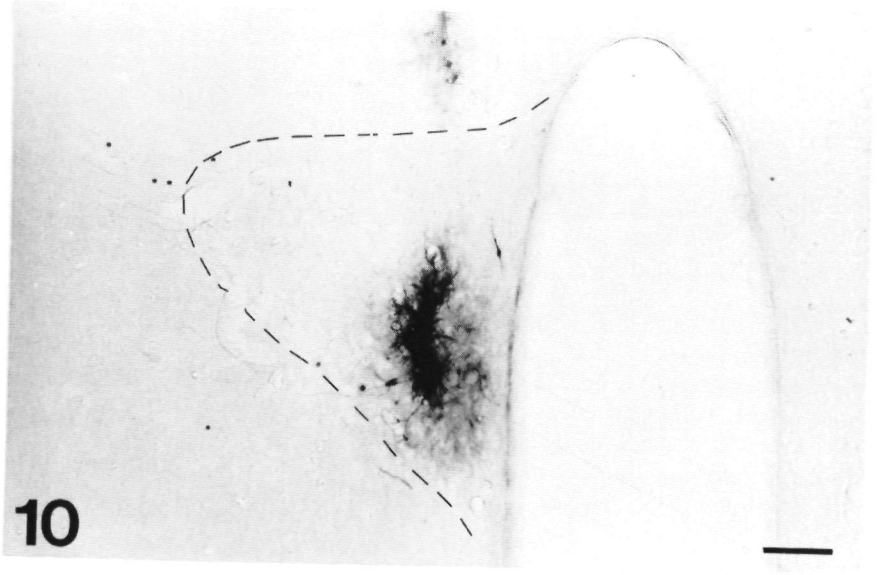
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from the caudal part of the DHA (R148).

Many terminals from zona incerta efferents are found in the dorsolateral CG and peripheral part of the ventrolateral CG. Caudally, zona incerta fibres are positioned in the medial parts of the pontine central gray (CGPn), medially and laterally from the postero-dorsal tegmental nucleus.

In addition to the CG, efferent hypothalamic fibres running through the dorsal brainstem fibre stream, innervate various mesencephalic nuclei within or close to the mesencephalic and pontine central gray (CG and CGPn) (Fig.3). Further caudally, efferent fibres from the HGA cluster in the ventrolateral corner of the fourth ventricle, running caudally towards the nucleus of the solitary tract (see below). A large number of PHA-L labelled fibres and oxytocinergic fibres in this area is positioned perpendicular to the wall of the ventricle (Figs. 5-7H). Fibres running within this fibre stream from injection sites outside the HGA are only sparsely found caudal to the pontine central gray (Fig.8H).

The **locus coeruleus** (LC) is only moderately innervated by DHA and PVH neurons (Fig.5H, 6H), but it receives a large number of varicose oxytocinergic fibres (Fig.7H). Injection sites in the anterior hypothalamic area and intermediate hypothalamic area (R207, R153) revealed only a sparse number of fibres in the LC. The injections in the zona incerta (R140, R293) and caudal part of the dorsal hypothalamic area (R 148) revealed no labelled fibres in the LC.

The **nucleus of Barrington** (Bar) is moderately innervated by DHA fibres in most, but not all, DHA series. No oxytocinergic fibres are found in this nucleus. Except for series R140 and R148, hypothalamic areas outside the HGA project to Bar as well.

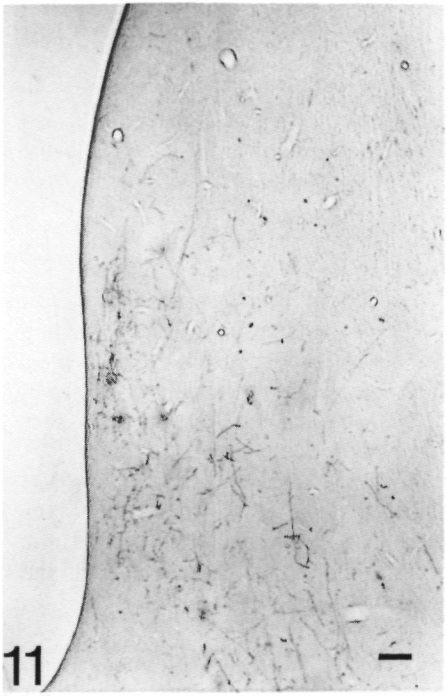
A large number of varicose fibres from the DHA enters the **lateral parabrachial nucleus** (LPB). The **medial parabrachial nucleus** (MPB) is only moderately innervated by the HGA (Fig. 5H). PVH fibres innervate the LPB and MPB as well. A large number of oxytocinergic fibres is found in the LPB. In control series only a few labelled fibres are found in the parabrachial nuclei (Fig.8H). No fibres from the zona incerta are found in this region (Table 1).

A considerable number of DHA fibres and terminals is found in the **nucleus of the solitary tract** (NTS) and the **dorsal motor nucleus of the vagal nerve** (DMV) (Fig.5I). The NTS and DMV are also densely innervated by varicose PVH- and oxytocinergic fibres. Many labelled terminal arborisations are found as well in these nuclei (Fig.6I, 7I). Except for case R112 all injection sites within the HGA, together with one injection site in the caudal DHA (R148) resulted in labelled fibres in the NTS and DMV. Our other hypothalamic injections did not result in labelled fibres in these nuclei.

Ventromedial brainstem fibre stream.

This fibre stream is positioned in the ventromedial parts of the brainstem and innervates the ventral tegmental area (Fig.3). Further caudally, this fibre stream innervates the raphe magnus.

The **ventral tegmental area** is heavily innervated by fibres from injection sites within the HGA, but only sparsely by fibres from hypothalamic areas outside the HGA (Figs.3, 5-8F). Fibres from the HGA have many varicosities in this area, while fibres from control injections are mainly smooth. Within the ventral tegmental area (VTA), a large number of highly varicose fibres from neurons within the HGA, as well as oxytocinergic fibres, turn laterally towards the ventral aspects of the central tegmental field (CTF),



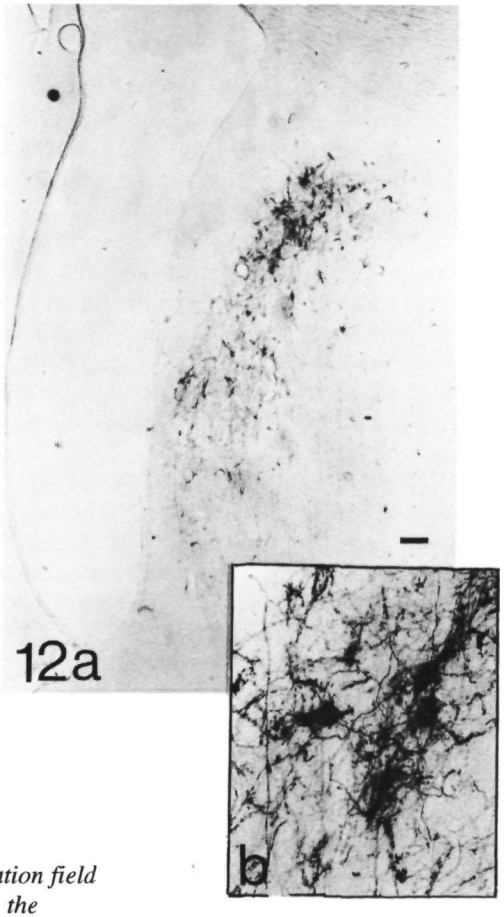


Figure 11 *Photomicrograph of termination field in the septum after a PHA-L injection into the dorsal hypothalamic area (R146). Scale Bar: 100 µm.*

Figure 12 *A: Photomicrograph of PHA-L immunoreactive "pericellular baskets" in the dorsolateral septum after PHA-L injection into the intermediate and anterior hypothalamic area (R207). B: Detail of Fig. 12A.*

joining the fibres of the lateral descending fibre stream of the medial forebrain bundle (Figs.3, 5F, 6F). Some fibres turn dorsally in the median plane towards the median and dorsal raphe nuclei (Fig.3). These fibres are smooth with only a sparse number of varicosities.

The **dorsal raphe nucleus** is moderately innervated by DHA fibres. No fibres from the PVH have been found innervating the DR. Oxytocinergic and non-HGA fibres innervate the DR only sparsely. A moderate amount of fibres from the DHA project to the **median raphe nucleus**. No PVH and oxytocinergic fibres have been found in the MnR. Control injections resulted in only a sparse number of labelled fibres in the MnR.

Caudal to the central tegmental field, the ventromedial descending fibre stream from the hypothalamic sites outside the HGA is very much diminished. From control injections, labelled fibres in the raphe magnus were only found in cases R153 (AHA

injection) and R148 (caudal DHA injection).

A moderate number of DHA fibres, but only a small number of PVH fibres are found in the **raphe magnus** (RMg) (Figs.5,6I). Hypothalamic areas outside the HGA appear to project only sparsely to the raphe magnus (Fig.8I, table 1). Many oxytocinergic fibres are found in the RMg (Fig.7I).

Ventrolateral brainstem fibre stream.

Fibres from the ventrolateral brainstem fibre stream innervate the ventral aspects of the central tegmental fiels, the ventrolateral tegmental area (VLTg), the noradrenergic A5 area and the ventrolateral medulla (VLM) (Fig.3).

Some fibres within the ventral part of the **central tegmental field** (CTF) turn dorsally along the medial side of the lateral lemniscus (ll) and appear to innervate the **nucleus tegmentalis pedunculopontinus** (PPTg) (Fig.3). Other fibres stay in the ventral parts of the tegmentum descending towards the medulla oblongata. Injections in the DHA resulted in either sparse (R112, R172) or large (R146, R111) number of labelled fibres in the **noradrenergic A5 region**. The origin of this variation could not be determined. The smaller number of labelled neurons in the series revealing sparse fibres in the A5 region as compared to the number of labelled neurons in the series revealing large A5 innervation could have contributed to this difference. A large number of PVH fibres is localized in this area (Fig.6H). After control injections, projections to this region is found only in the caudal DHA injection (R148) and the zona incerta injection (R293). Many fibres and terminals from this zona incerta injection are found in the pontine region dorsal to the medial lemniscus.

Caudally, DHA fibres cluster in the ventral parts of the medulla in the **lateral reticular nucleus** (LRt) and **ventrolateral medulla** (VLM). Some fibres turn dorsally and innervate the nucleus of the solitary tract (Fig.3). PVH fibres innervate the ventrolateral medulla (VLM) as well (Fig.6I). An extensive oxytocinergic innervation of the ventrolateral medulla is found (Fig.7I).

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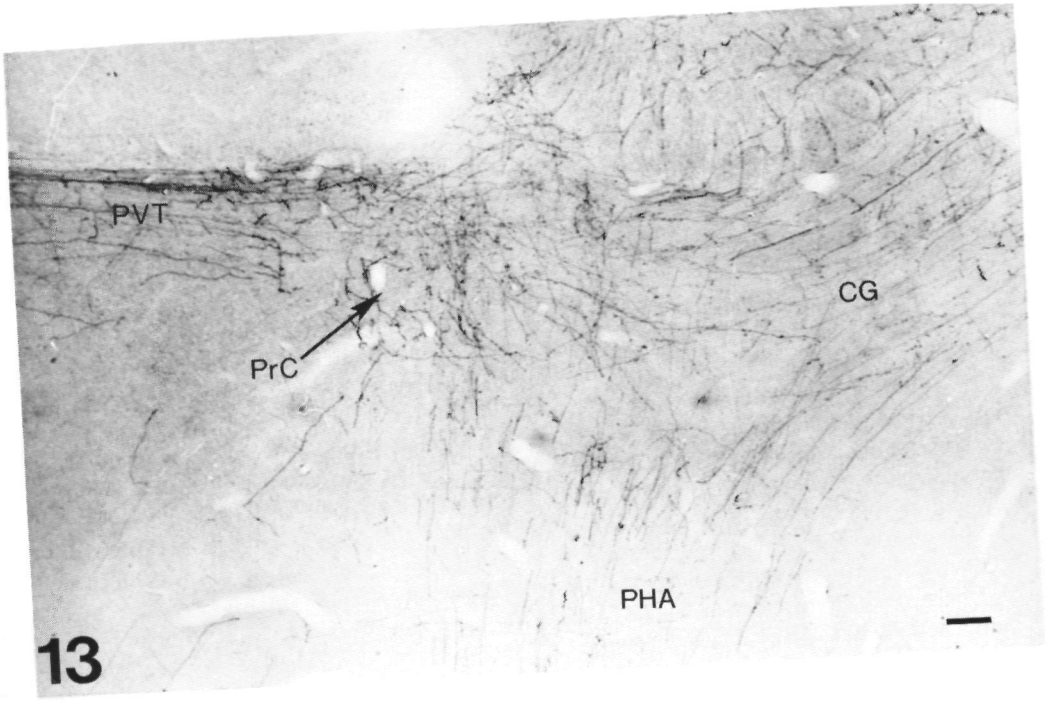
Figure 13 *Fibres at the transition between diencephalon and mesencephalon after a large PHA-L injection into the PVH/ DHA/ AHA area (R288). Note the crossing of the fibres from the dorsal thalamic fibre stream and from the dorsoventral descending fibe stream and the large number of terminals in the precomissural nucleus. Scale bar: 100 μm.*

Figure 14 *Photomicrograph of immunoreactive oxytocinergic neurons in the rostral hypothalamus. Scale bar: 100 μm.*


PVT
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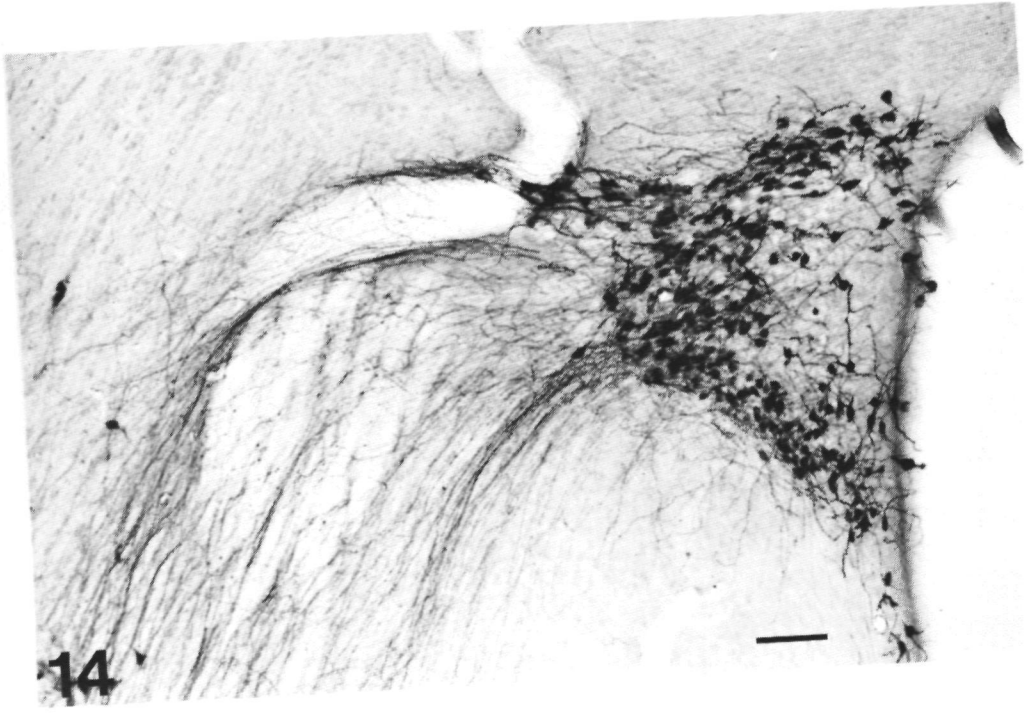
PHA
13


14

Discussion.

Technical remarks.

The anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) has been used to determine the efferent pathways of the hypothalamic "grooming area" (HGA). An advantage of this method is the complete labelling of neurons in the injection site [26]. The HGA can not be regarded as a cytoarchitectonically defined area and the neurons in this area that are involved in grooming behaviour have not been identified in some other, more specific way. Therefore, a precise localization of labelled neurons in the description of efferent projections is necessary. The combination of eliciting behaviour by local injection through implanted cannulae in freely moving animals together with a subsequent iontophoretic PHA-L injection through the guide cannula has been attempted. However, since the percentage of successful injections using this method was very low, this combined procedure was abandoned. Instead, we first carefully delineated the area, where the largest number of positive injection sites were found (Fig.1). Subsequently, a number of PHA-L injections in this area have been made in a separate series of rats and their efferents have been compared with those of injections outside this hypothalamic "grooming area".

The hypothalamic grooming area has been delineated using the previously reported successful injection sites [47,80,81,100]. Although the extent of the area of activated neurons after a single injection could not be observed directly, the results of subsequent injections in the same animals, varying 0.5 to 1.0 mm in depth, together with a detailed plotting of the positive and negative injection sites, suggested that a single injection only activated a small area [80]. A hypothalamic "grooming area" could therefore well be delineated using the positive and negative sites as markers [80]. In addition, this hypothalamic grooming area is identical for different stimulation techniques [47,81,100]. The HGA is positioned in the dorsomedial part of the hypothalamus and consists of parts of the hypothalamic paraventricular nucleus (PVH) and the dorsal hypothalamic area (DHA).

Efferent fibre streams from the hypothalamus and their characteristics.

The PHA-L-labelled fibres in the present study, originating from several populations of neurons in or surrounding the hypothalamic "grooming area" (HGA), did not form well determined bundles or fascicles, but rather spread out diffusely throughout the hypothalamus, clustering in some brain areas and often intermingling with each other, thus forming diffuse fibre "streams". All along the course of the fibres, many varicosities were apparent, although there were differences in the number of varicosities in different areas of passage. These varicosities may contain synaptic specializations [106] or may communicate by non-synaptic exocytosis [12,13]. Therefore, information transfer may occur in many brain sites that are traversed. The efferent fibres from the hypothalamic areas as described in the present study can therefore be considered to form "open pathways", i.e. pathways which have the potential for influencing brain sites throughout their extent, rather than only in their area of final termination [64].

Although the pattern of efferent fibre "streams" was largely similar for all hypothalamic injection sites, which have been studied, differences in the number of fibres in each stream following different injections could be distinguished. Within the pattern of efferent fibres, three major ascending streams, a dorsal, a lateral and a ventromedial

ascending stream, two thalamic fibre streams, a dorsal and a ventral thalamic fibre stream, and three major descending fibre "streams", a dorsal stream, a ventromedial and a lateral descending stream, could be identified. These small streams form only a minor efferent output pathway of the HGA. Within the hypothalamus, the largest number of descending fibres was situated medial to the medial forebrain bundle [31,63]. Further caudally, descending fibre streams were found in caudal extensions of the medial forebrain bundle in the ventral parts of the brainstem, in the fasciculus longitudinalis dorsalis of Schütz in the central gray, and in the dorsal part of the medulla oblongata [31,64].

Efferent projection sites: specificity for the HGA.

The aim of the present study was to investigate 1) differences in efferent projections between the HGA and other hypothalamic areas surrounding the HGA, and 2) the similarities in efferent projections of different parts of the HGA. The distribution of oxytocinergic fibres was taken as an example of a peptidergic system, that is possibly related to grooming behaviour [20,37,38,68,101].

The various hypothalamic areas that have been studied, all project to a large number of brain areas (see table 1). These common efferent projection areas may be indicative for the fact that different behavioural responses use the same functional systems, like visceromotor, endocrine, skeletomotor, and limbic functions. Whether these brain areas are inhibited or excited may be dependent on the type of behaviour that is displayed.

In general, a strong similarity in the efferents of those parts of the PVH and the DHA, that together form the HGA, has been found. Within the efferents of the hypothalamic areas that have been studied, a number of projection areas appear to be innervated preferentially by fibres from the HGA. Other brain areas receive efferent projections from both HGA and hypothalamic areas surrounding the HGA. In some of these loci a specific spatial organization of terminal fields is present.

Rostrally, in the anterior part of the medial preoptic area, a preferential HGA projection was found in the **anterior periventricular nucleus** (PeAV), close to the organum vasculosum of the lamina terminalis (OVLT) and in the **median preoptic nucleus**. The innervation of the OVLT by the PVH has been reported before [50], but it now appears to originate from the whole HGA.

Caudally, the innervation of the **mesencephalic ventral tegmental area** (VTA) is more or less specific for HGA fibres. Hypothalamic areas surrounding the "grooming area" only sparsely project to the VTA. In addition, these fibres reveal no or only sparse varicosities. The ventral part of the **central tegmental field** (CTF), dorsal to the lateral part of the substantia nigra, appears to receive a preferential HGA innervation as well. Innervation of the VTA and CTF by PVH neurons has been reported before, but it was concluded that fibres entered the CTF through the zona incerta [55]. Our results suggest, however, that fibres enter this part of the CTF either directly through the lateral descending stream or through the ventral descending stream, turning laterally at the level of the VTA.

The nucleus **raphe magnus** is also preferentially innervated by HGA fibres. Efferent connections from neurons the HGA to the RMg have been demonstrated before [31,103]. However, the large innervation of the raphe magnus by zona incerta efferents, as reported by Veening et al. [103] is not found in our study. Raphe magnus innervation from

the zona incerta may arise from more lateral and caudal aspects of the zona incerta [103].

The **lateral parabrachial nucleus** (LPB) is another area that is preferentially innervated by neurons from the HGA. Hypothalamic innervation of the LPB has been reported before to arise preferentially from the HGA and the lateral hypothalamic area (LHA) [60,61]. In te present study, the latter projections could not be confirmed, since no control injections were made in the LHA.

A similar strong projection from the HGA has been found for the **peduncolopontine tegmental nucleus** (PPTg) and the **ventrolateral medulla**. PVH innervation of the ventrolateral medulla was reported before [55].

In the lower brainstem, preferential HGA projections from both DHA and PVH neurons were found in the **nucleus of the solitary tract** (NTS) and **dorsal motor nucleus of the vagal nerve** (DMV). A large innervation of oxytocinergic fibres has been observed in this area as well. These findings are in agreement with other reports on PVH- and oxytocinergic efferents [54,55,88,92,93,96], but their origin seems to include the whole HGA. PHA-L-injections in sites surrounding the HGA did not result in PHA-L labelled fibres within the NTS.

Spatial organisation of septal and central gray innervation.

In addition to brain areas that are preferentially innervated by neurons within the HGA, brain areas have been found with a spatial organisation of hypothalamic efferents.

The **lateral septum** receives a large innervation by DHA neurons [18,41,53,74]. The existence of PVH projection to the septum has been controversial: Some HRP [53,92] and electrophysiological [62,69] studies demonstrated such efferents. However, other reports failed to find such connections [18,84]. Our results from small injections that are restricted to the parvocellular PVH reveal that at least a small population of PVH neurons project to the septum.

Dorsal hypothalamic area efferents appear to cluster in the ventral part of the lateral septal nucleus, close to the lateral ventricle. Although efferents from the hypothalamic paraventricular nucleus and from the oxytocin-containing neurons in the hypothalamus innervate the septal area only moderately, these fibres tend to concentrate in the same ventral part of the lateral septal nucleus. The same pattern of oxytocinergic innervation of the lateral septum was shown to exist in the garden dormouse [30]. Corticotropin releasing hormone (CRH) terminals concentrate in this area as well [84]. This CRH innervation of the septum appears to originate from the dorsal and the anterior hypothalamic areas, rather than the PVH [84]. Hypothalamic areas surrounding the "grooming area" do not innervate the ventral part of the lateral septal nucleus in such a specific way. The ventral part of the lateral septal nucleus therefore seems to receive a preferential projection from the hypothalamic "grooming area".

All parts of the hypothalamus that have been studied project extensively to the mesencephalic **central gray** (CG), as might be expected from previous studies [3,31,54,55,56,96,-103,104]. Within the CG, a spatial organisation of hypothalamic fibres is found. Fibres from the "grooming area" are organised in a rostrodorsal and a caudal ventrolateral cluster. These clusters tend to remain in the central part of the CG, surrounding the aqueduct. This spatial organisation of hypothalamic efferents confirms previous reports [3,104].

Non-specific innervation.

Many brain areas were found to be innervated by both HGA neurons and non-HGA neurons, without spatial or numerical differences between HGA and non-HGA projections.

The innervation of the amygdala from the hypothalamic "grooming area" is only small and tends to be restricted to the medial parts of the amygdala including the **medial amygdaloid nucleus**. This area was also innervated by neurons surrounding the HGA. The presence of PVH efferents to the amygdala has been suggested by electrophysiological experiments [69]. Our results show, that PVH fibres do not innervate the **central amygdaloid nucleus**. We found in agreement with previous reports [4,19,102], that other parts of the hypothalamus project to the amygdala as well.

Fibres terminating in the **thalamus** could be observed in all injections studied, in accordance with other reports [16,22,28,65]. In sagittal sections, the rostrocaudal direction of the fibres within the dorsomedial part of the thalamus is clearly visible. Although no detailed analysis of the thalamic innervation has been carried out in the present study, some differences in thalamic innervation by different hypothalamic areas could be distinghuished. In particular, the strong innervation of the mediodorsal thalamic nucleus by the intermediate hypothalamic area injections was evident.

In the transition area between diencephalon and mesencephalon, a large number of terminals and varicosities was found in the **precommissural nucleus**. Fibres from the thalamic stream cross the fibres of the dorsomedial hypothalamic stream in this area and turn towards the mesencephalic central gray. Fibres originating from different hypothalamic areas positioned within the fasciculus retroflexus running towards the interpeduncular nucleus are only very sparse, in contrast to other reports [11].

Within the hypothalamus the sparse innervation of the ventromedial hypothalamic nucleus (VMH) from the "grooming area" is remarkable. PHA-L injections restricted to the parvocellular PVH showed no VMH innervation at all. This is not in agreement with the report by Luiten et al. [54], but the injection site shown in their study was much larger than in our study and could have included other parts of the PVH. Oxytocinergic fibres entering the VMH are only sparse. Taken together, it appears, that fibres from the "grooming area" circumvent the VMH rather than innervate it. However, since neurons from the VMH are known to spread their dendrites outside the core of the VMH [59], it is possible that fibres from the HGA contact VMH dendrites outside the borders of the VMH, in or even outside the shell of that nucleus.

Putative grooming relevant circuitry.

Grooming behaviour has different functions, like temperature regulation, maintenance of the condition of the fur and normalising arousal levels after stressful events [35,97a]. The structure of grooming behaviour elicited in different circumstances differs [14a,83]. Intracerebroventricular injection of various neuroactive peptides have been reported to elicit different aspects of grooming behaviour [20,21a,27,75,101]. Even different stimulation techniques applied to the same brain area can elicit differences in the structure of the elicited grooming behaviour [100]. Electrical stimulation of the hypothalamic "grooming area" appears to increase grooming and to reduce scratching, while ACTH injections elicit an increase in both grooming and scratching [100]. NMDA injections into the "grooming area" can alter the structure of the elicited grooming response as well (Chapter 2.5).

Detailed analyses of the fine structure of grooming behaviour as it can be elicited by manipulation of brain areas are therefore seriously needed.

It has been suggested, that the brainstem itself is able to perform a large number of behavioural responses without the influence of higher cerebral structures [5]. Although chronically decerebrated rats were fully capable of keeping a clean fur [6] and responded to water spraying of the face and body by vigorous grooming similar to control animals [8], the sequential organisation of grooming behaviour in decerebrated animals was severely disturbed [6]. It was concluded that the neuronal circuitry generating basic patterns of grooming behaviour is not organized in discrete centres, but consists of a diffusely organized network in the hindbrain [5,6,8]. It therefore seems likely, that hypothalamic grooming area exerts its control on basic behavioural patterns, that are primarily generated at the level of the brainstem.

The role of **telencephalic structures** in the regulation of grooming behaviour has only been sparsely investigated. Box and Mogenson [9] reported an impairment in grooming behaviour after **corticomedial amygdala** lesions. This was not confirmed by Kimble and Godding [40]. However, their lesions appear to have been placed more laterally and ventrally in the amygdala than those of Box and Mogenson, thus sparing the central amygdaloid nucleus and parts of the medial amygdaloid nucleus [40]. Like the amygdala the role of the **septum** in the regulation of grooming behaviour has never been thoroughly investigated. Altman and Wishart [1] reported the elicitation of grooming and feeding behaviour during and after electrical stimulation of the septum. They suggested that these behaviours were elicited due to disturbance by septal stimulation of the homeostatic systems that are regulated by the hypothalamus [1]. The present study clearly shows that the HGA has a marked efferent projection to the septal area. This projection may be involved in motivational aspects, that are of importance during the proper performance of an integrated grooming response.

Although grooming behaviour was elicited by bombesin injections into the **hypothalamic ventromedial nucleus** (VMH) [46], on the basis of the efferents from the "grooming area" it is not likely that the core of the VMH is involved in the regulation of grooming behaviour elicited by DHA/ PVH stimulation. It is, however, possible that fibres from the PVH contact dendrites of VMH neurons positioned within the shell of the VMH [59]. In a study using electrical stimulation to elicit grooming behaviour, some positive stimulation sites were found in the small zone between VMH and dorsomedial hypothalamic area (DMH) [47,80] and not within the VMH itself. Grooming induced by bombesin injections could possibly be elicited by stimulation of this small zone.

The mesencephalic **central gray** (CG) has also been reported to be involved in the regulation of grooming behaviour [23,46,89,90]. ACTH injections into this centre elicit grooming behaviour, while lesions of the CG attenuate grooming behaviour induced by intracerebroventricular ACTH injections [90]. ACTH fibres in the CG are concentrated in the ventral parts of the CG [13,82,104] (Roeling, personal observation). The efferents of the hypothalamic "grooming area" are concentrated in this part of the CG as well. Neuronal stimulation of this part of the CG by excitatory amino acids induces hypotension [14]. This effect appears to correlate with the general role of grooming in dearousal after stressful events [35].

Local injections of oxytocin, CCK, or α-MSH into the **ventral tegmental area** (VTA) induce grooming behaviour [37,98]. The VTA is part of the mesolimbic dopaminergic system and projects to the nucleus accumbens [95]. It has been suggested that the balance in

activity of the mesolimbic and the nigrostriatal dopamine systems is involved in the control of grooming behaviour [15]. The striatum has also been reported to be involved in the sequential patterning of grooming components [7].

Grooming behaviour has been elicited by electrical stimulation of the **ventrolateral medulla** in cats (A5 area) [5,6]. The ventrolateral medulla is inter alia involved in cardiovascular regulation [21]. This is interesting, since electrical and chemical stimulation of the PVH have also been reported to elicit cardiovascular responses, either as an increase [32,71,72] or as a decrease in heart rate or blood pressure [29,38,107]. Jin & Rockhold [32] reported phasic increases in blood pressure and heart rate concomitant with grooming behaviour after infusion of low doses of kainic acid into the PVH of freely moving animals. The connection of the hypothalamic "grooming area" with the ventrolateral medulla may be involved in this double effect.

Grooming has been elicited by means of intracerebral electrical stimulation in various brainstem areas in rat and cat [2,5,6,58]. The pattern of grooming behaviour elicited by brainstem stimulation apparently could be adapted to peripheral stimuli, like the presence of dirt particles and their position in the fur [6].

The **nucleus of the solitary tract** (NTS) is considered to be an important recipient of peripheral sensory information, since most of the visceral sensory input converges in the NTS [51]. Injection of bombesin into the NTS has been reported to elicit grooming, and, more specifically: scratching behaviour [33,34].

Ventricular system.

Many PHA-L labeled fibres, originating in the HGA, but also in hypothalamic areas outside the HGA were found in close approximation of the ventricular system, e.g. along the lateral ventricle within septal regions, along the third ventricle within ventral hypothalamic regions near the arcuate and periventricular nuclei, along the aqueductus cerebri within the central gray, and along the fourth ventricle, dorsal to the locus coeruleus. The fibres were not only running parallel to the wall of the respective ventricles, but many were running perpendicular to these walls. These fibres appeared to be positioned in between the ependymal cells. The function of these fibres is at present unknown, especially since we can not conclude from light microscopic observations, whether or not the fibres contact the ventricular surface itself. Interestingly, a large number of studies has been performed on grooming behaviour elicited by intracerebroventricular injection of various neuroactive substances, like ACTH, bombesin, oxytocin and vasopressin [20,27,68,75-,76,101]. These studies suggest that the cerebrospinal fluid is capable of transporting information and may activate brain areas that are involved in the regulation of grooming behaviour. A number of brain areas, among them the PVH, have been reported to be able to take up HRP from the ventricular system and may therefore have access to information derived from the ventricular fluid [10].

Concluding remarks.

The study of the neural circuits underlying the organization of behaviour is important for our understanding of the mechanisms underlying behavioural response of an animals to a variety of external and internal stimuli. The fact, that stimulation of hypothalamic sites can elicit a large number of rather complete behaviours together with associated autonomic changes suggests, that the hypothalamus is an important part of the circuit underlying behaviour [36,42,44,66,85,94,97,106]. Since the induced types of behaviour may consist of initiation, procurement and consummatory phases, it is conceivable, that the hypothalamus is involved in the motivational aspects of behaviour [94]. The many efferent connections of the hypothalamus to limbic and brainstem structures indicate, that the hypothalamus may play an important role in preparing and activating a large number of brain areas that are involved in the execution of the required behavioural response [94,97]. Within the hypothalamus, a spatial organisation of "behaviourally determined areas" is apparent [47,48,49]. The study on the efferent connections of specific hypothalamic areas that have been shown to be involved in the elicitation of specific behaviours can provide an anatomical basis for future studies on brain mechanisms that are involved in the regulation of hypothalamically induced behaviours. Finally, new anatomical techniques crossing the borders between physiology, behaviour and anatomy, like c-fos methods and deoxyglucose techniques, may be useful tools in further studies on the brain circuitries and mechanisms involved in the regulation of behaviour [57,70,73,79,85].

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PART 3 HYPOTHALAMICALLY ELICITED ATTACK BEHAVIOUR

3.1 INTRODUCTION TO THE STUDIES ON HYPOTHALAMICALLY ELICITED ATTACK BEHAVIOUR.

In part 2 of this thesis, some aspects of the neuronal substrate underlying hypothalamically elicited grooming behaviour have been presented. The hypothalamic "grooming area" has been delineated, the structure of grooming behaviour elicited by stimulation of the "grooming area" has been described and the efferents of the "grooming area" have been studied. In order to assess whether general conclusions can be drawn concerning the neuronal substrate of hypothalamically elicited behaviour, a study on attack behaviour has also been undertaken. The study of attack behaviour elicited by electrical stimulation of parts of the hypothalamus has been a main issue for a number of years in our group [1,2,3,4,5]. In general, the same approach has been followed as for investigating grooming behaviour: after determining whether neuronal cell bodies are involved, the description of the efferent connections of the hypothalamic "attack area" is presented, which will be compared with the efferent connections of hypothalamic areas outside the "attack area". First, some general information will be presented about the brain areas involved in agonistic behaviour in the rat (chapter 3.2). Since attack behaviour can only be performed in a social environment, we had to develop a new cannula system that allows local injections in brain areas without disturbing ongoing social interactions (chapter 3.3). The results of stimulation of the ventral parts of the hypothalamus, in and near the hypothalamic attack area, are presented in chapter 3.4. The efferent connections of the hypothalamic attack area, the specificity of these efferents for the attack area and the relation of the hypothalamic attack area with other "aggression associated" brain areas will be presented in chapter 3.5.

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3.2 AGGRESSIVE BEHAVIOUR.

3.2.1 Definition.

Aggressive behaviour and agonistic behaviour have been defined in a number of ways [36,49]. In general, the term "agonistic behaviour" is used for the whole set of behavioural elements that can be displayed by an animal towards a conspecific, with the exception of sexual behaviour. Therefore it includes offensive (or aggressive) behaviours, like attack and threat, as well as defensive behaviour and flight [49]. In both offensive and defensive behaviours, similar behavioural elements may be present [36]. In this brief introduction, the emphasis will be placed on the brain areas, that have been suggested to play a role in the regulation of the offensive type of agonistic behaviour, mainly attack behaviour. However, it is necessary to include some other types of agonistic behaviour, since experimental settings may result in behaviour, which includes both offensive and defensive elements. Since the neuronal mechanisms regulating agonistic behaviour in cats and rats may differ [36], I confine myself here to what is known about the neuronal mechanisms regulating agonistic behaviour in rats. For a review on the extensive studies of agonistic behaviour in cats the reader is referred to e.g. Siegel & Pott [50].

3.2.2 Types of aggression.

In the studies on the neuronal regulation of attack behaviour displayed by male rats, a number of experimental settings have been used, e.g. territorial aggression, predatory aggression and pain-induced fighting.

Territorial aggression is displayed in a colony situation or in a resident-intruder paradigm. Within a resident- intruder paradigm, animals in home cages are tested for aggressiveness towards a strange conspecific, that is introduced in the animals home cage. There is a high incidence of offensive behaviour from the side of the resident animal and the outcome of the fight is nearly always in favour of the resident. In colony situations, a similar diadic interaction is displayed between the dominant male and the intruder [15].

Predatory aggression can be defined as the killing of a non- conspecific in order to eat the victim. This type is therefore suggested to be closely related to feeding behaviour. In rats, killing of non- conspecifics has been tested on mice, frogs, turtles and locusts. Mouse killing in this respect seems to be somewhat different, since only few rats kill mice, while a large majority of rats usually kill frogs and turtles [9]. In the studies on mouse killing, selection lines of killer and non-killer rats have been used [5,19].

Pain-induced aggression can be elicited by delivering electric shock pulses to either footsole or tail of mice and rats, thus inducing an aggressive response. Because of the characteristics of this type of aggression, shock-induced fighting is regarded as a form of

defensive aggression [15].

3.2.4. Brain areas involved in agonistic behaviour.

Experiments using electrical stimulation of specific brain areas have been very useful in the study of the neuronal correlates of attack behaviour. Lesioning studies have contributed as well. Local injections of neuroactive substances have been used only sparsely.

Forebrain structures

Lesioning the septal regions results in a type of behaviour, that is referred to as septal rage [4]. Characteristic are the unprovoked attack on experimenters and the high reactivity to external stimuli [4]. Within the septum, the anterolateral region seems to be a crucial part that has to be destroyed in order to get the typical behavioural change [2,3,48]. Lesions in the area between the accumbens and the medial portion of the preoptic area induce rage as well [2,3]. This area may include the subcommissural part of the substantia innominata (SIC), as well as the lateral part of the preoptic area (LPOA) [2,26]; Diagonal band lesions as well as accumbens lesions appeared to be ineffective [2,3].

The behavioural characteristics of animals with septal lesions were regarded by Albert and collegues as dissimilar to behaviour seen in territorial situations and to aggressive behaviour elicited by electrical stimulation of hypothalamic sites [4,6,7]. In a diadic encounter, the rats with septal lesions attacked conspecifics. However, no piloerection or lateral threat were seen, indicating that both overt behaviour and autonomic activation differ between lesioned animals and control animals in a territorial situation [3,5]. Moreover, lesioned rats that were introduced in a colony, did not attack and, when tested in home colonies with an intruder, often broke off an initiated attack upon the intruder [6]. It was therefore concluded that septal lesions did not enhance intraspecific social aggression, but increased defensiveness [6].

Both the bed nucleus of the stria terminalis (BNST) as well as the medial forebrain bundle were reported to be involved in territorial aggression as well, but not in mouse killing [13]. The BNST was also reported to be involved in the aggression occurring in a food competition test [39].

The amygdala is a heterogenous structure and it is therefore likely, that different amygdaloid structures are differently involved in the modulation of behaviour. Lesioning the corticomedial amygdala resulted in a decrease in approach and lateral threat, but not in upright posture. This indicates that these lesions may affect the offensive component of the behaviour [31]. Amygdala lesions that were placed lateral to the medial amygdaloid nucleus and included basolateral and central amygdaloid nuclei did not result in changes in interspecific aggression, but reduced defensiveness towards a predator [16]. Defence towards experimenters was reduced after cortical and central amygdaloid nucleus lesions [29]. Using a food competition test with amygdala lesioned animals, Miczek et al. [39] showed the involvement of the periamygdaloid cortex and the cortical amygdaloid nucleus in offensive behaviour. The medial amygdaloid nucleus was suggested to be involved in social learning

processes and in aggressive behaviour [17,53]. Within the medial amygdala, serotonin plays a role in the suppression of both mouse killing behaviour and shock induced (defensive) fighting [47]. This serotonergic inhibition was suggested to originate in the dorsal raphe nucleus [47]. Within the amygdala, the medial amygdaloid nucleus (MeA), and especially its posterior part, projects most extensively to hypothalamic structures that are involved in aggressive behaviour [38]. Although this may indicate that the posterior part of the MeA is more involved in aggressive behaviour than the anterior part, a recent report has indicated that the rostral part of the MeA is specifically active in the consummatory phase (actual attack) of territorial aggression in hamsters [46].

The prefrontal cortex (PFC) in rats has been subdivided in two parts: the orbital and the medial PFC [37a]. The orbital PFC is reported to be involved in the regulation of agonistic behaviours, in particular shock-induced fighting [30]. Dopaminergic deafferentation of the orbital PFC resulted in an increase in lateral threat, indicating the possible inhibitory role of dopamine in the prefrontal cortex [18]. The dopaminergic innervation of the PFC is mainly originating in the area A10 (ventral tegmental area). The prefrontal cortex was proposed to exert its inhibitory role in agonistic behaviour in part by its connections with hypothalamic sites that are known to be involved in attack behaviour, since electrical stimulation of both PFC and hypothalamus resulted in an increase in threshold current necessary for the elicitation of hypothalamically induced attack [18].

Hypothalamus

The role of the hypothalamus in the regulation of attack behaviour was proposed after the initial experiments of Hess using electrical stimulation of parts of the hypothalamus in the cat [27]. In the rat, lesioning the lateral hypothalamic area resulted in the attenuation of territorial aggression [1]. Electrical stimulation of this area resulted in a facilitation of mouse killing behaviour [45,57] and in aggression towards conspecifics [45]. Panksepp [45] recognized three hypothalamic regions where attack behaviour can be elicited: 1: the lateral hypothalamic area at the caudal end of the optic chiasma and overlying the optic tract; 2: the perifornical and far lateral hypothalamic area at the level of the ventromedial nucleus and 3: the ventral hypothalamus at the lateral border of the ventromedial nucleus. Each area elicited different types of attack towards alive or dead mice and rats. Differences in stimulation induced aversion and reward could also be determined [45]. Panksepp therefore stated, that the division of attack behaviour into quiet biting attack and affective attack, as was distinguished in cats [50], could also be made in rats [45]. These findings were based on relatively few electrode placements and although these results were confirmed to some extent by other reports (e.g. [31]), later more extensive and systematic discrimination studies suggested that these responses derive from the same neural system [33,34,36,37]. Differences between behavioural responses were attributed to variations in stimulus intensity, posture of the opponent and simultaneous activation of different behavioural systems [36]. The hypothalamic locus from which attack behaviour can be elicited is situated in the area ventral to the fornix and includes the ventrolateral pole of the ventromedial hypothalamic nucleus (VMH) [33,34,37]. The attack patterns displayed by individual animals by stimulation of individual electrode placements are very rigid and strain-specific [33,35]. Like in territorial aggression, the attack is mainly directed to the head and neck region of the victim [33], although strain differences occur [35]. Interestingly, sideways threatening, which is conside-

red to be an "ambivalent" posture, was never seen in hypothalamically elicited attack [33,34,36].

The role of the ventromedial hypothalamic nucleus (VMH) in the inhibition of parts of the agonistic repertoire was proposed after lesioning experiments, that increased threatening postures of males in a territorial setting [44]. However, further studies indicated, that these changes in behaviour could be due to the involvement of the ventral premammillary nucleus, rather than the posterior VMH [52].

Lesioning the anterior hypothalamic area resulted in an increase in defensive fighting [44]. Lesions in the anterior preoptic area reduced territorial aggression, but did not affect mouse killing, suggesting that these behaviours did not use a common substrate in the preoptic area [13].

Thalamus.

Cholinergic stimulation of the mediodorsal thalamus (MD) facilitated frog killing in natural killer rats [10]. The MD is proposed to exert its influence through descending connections to the midbrain central gray (CG) and dorsal longitudinal fasciculus of Schütz [10]. Some aspects of hypothalamically induced facilitation of predatory aggression may be dependent of thalamic activity [10]. The projection of the mediodorsal thalamic nucleus to the orbital prefrontal cortex could also be involved in the regulation of aggressive behaviour [18].

Brainstem.

In rats, the mesencephalic central gray matter (CG) seems to be more involved in defensive than offensive behaviour [12]. Yet attack behaviour can be elicited by electrical stimulation of parts of the CG [41]. Although the central gray is an important efferent projection area of hypothalamic structures involved in aggressive behaviour [38], destroying this area did not completely inhibit hypothalamically elicited attack behaviour and territorial aggression [42].

The ventral tegmental area (VTA) is suggested to be involved in predatory aggression, since cholinergic stimulation facilitates predatory aggression [11].

The locus coeruleus (LC). Lesioning the LC resulted in an increase in offensive behaviours in territorial aggression experiments [24].

The role of the dorsal and median raphe nuclei in aggression is controversial: lesioning these nuclei has been reported to result in an increase in predatory aggression [55] as well as a small decrease in territorial aggression [51].

3.2.3. Neuroactive substances.

The study on substances that are involved in the brain mechanisms of aggressive behaviour have largely been directed at aggression- inhibiting drugs. It is not our intention to summarize the different types of drugs, that have been found effective in this respect,

since it is largely unknown where and in what way many of these drugs are effective in their aggression-reducing properties. Therefore these studies have not contributed much to our understanding of the brain circuitries that are specifically involved in agonistic behaviour.

A seasonal rise in testosterone runs parallel with a rise in intermale aggression in many species. This rise in both testosterone and aggression peaks during the breeding season [31]. Gonadectomy, on the other hand, reduces aggressive behaviour. This reduction in aggressive behaviour can be restored after testosterone implants [31]. Androgens appear to influence hypothalamically elicited attack [14]. The site of action is possibly located in the subcommissural part of the substantia innominata, in the medial accumbens (lesioning studies) [8] or, partly, in the medial amygdala (castration and local infusion studies) [32].

In the study on agonistic behaviour, serotonin has evolved to be a major regulatory neurotransmitter [40]. Serotonergic drugs have been shown to inhibit mouse killing, to reduce brain stimulation- induced attack [36] and to reduce territorial aggression [43]. Serotonin depletion of forebrain structures results in an increase in territorial aggression and mouse killing [53]. However, the inhibitory role of serotonin in the regulation of aggressive behaviour has been questioned on the basis of other experiments. Lesioning the dorsal and median raphe nuclei may result in either aggression- enhancing or aggression- decreasing effects [51,55]. Local serotonin depletion in the amygdala causes a decrease in offensive behaviour in resident rats [25]. These contradictory results have been attributed to both strain differences and affective state differences [51].

Only few studies have been undertaken in order to assess the involvement of other neuroactive substances in the neuronal circuitry of aggressive behaviour. Corticotropin releasing hormone (CRH) has been reported to modulate some aspects of agonistic behaviour, after intracerebroventricular or amygdaloid injections [21]. Vasopressin may also be involved [21,32]. In hamsters vasopressin is also involved in the regulation of "hamster specific" agonistic behaviour (flank marking), possibly acting on neurons in the anterior hypothalamic area [22,23]. Since intracerebral infusion of GABAergic drugs elicit mouse killing and intraspecific attack [19,20], GABA may in some way or another be involved in aggression modulation.

In general, it can be stated, that only little is known about the neuronal circuitries underlying aggressive behaviour in the rat. Most of the studies on the involvement of different brain areas in agonistic behaviour have been performed using lesioning techniques, usually destroying large brain areas and fibre tracts. The involvement of different neuroactive substances in the regulation of various aspects of agonistic behaviour has been usually tested by peripheral application of the drug. The differences in experimental settings and the lack of detailed analyses of the elicited or modulated social interaction do in general not allow a detailed comparison of the experiments reported in the literature. Except for the many studies on hypothalamically elicited aggression and the sparse studies on stimulation of other brain areas, the involvement of other brain areas in aggressive behaviour has only been investigated in lesioning studies. Considerable gaps are therefore still present in our knowledge concerning the contribution of various parts of the brain in aggressive behaviour.

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3.3 A NEW MICROCANNULA FOR INJECTIONS IN RAT BRAINS WITHOUT DISTURBING SOCIAL INTERACTIONS.[1]

Abstract.

A new cannula system is presented that allows intracerebral microinjections of neuroactive substances in freely moving rats in a social environment. By connecting a microcannula to a freely rotating stainless steel spring, small (0.2 µl) injections can be made without disturbing the ongoing social interaction. This microinjection system can be used for instantaneous injections, but can also be used for microinfusions during a considerable longer period of time.

Introduction.

The study of the mechanisms in the brain that are involved in the regulation of behaviour is receiving increasing interest. It has become clear that various brain sites are involved in the regulation and coordination of different behaviours [14,15]. Direct influence on the activity of specific brain sites by means of electrical stimulation or chemical stimulation has proved a useful tool to study the role of brain sites in the regulation of behaviour [8,9,11]. Different techniques and equipment have been used for this purpose [4,6,7,13,16]. However, most of these cannula systems have shown disadvantages concerning the study of the role of brain sites in the regulation of behaviour in a non-stressed, social environment, since these techniques either require handling of the animal during the injection procedure [4], or do not permit the interference by a conspecific in the testing situation, due to the fragility of the cannula system [6,13,16].
The importance of studying animals in a social environment is specifically clear in the study on agonistic behaviour [9] and sexual behaviour [3,10,17]. It has become clear, that social partners in an experimental situation can have influence on the behavioural effects of local intracerebral injections in the experimental animal [5].

In the present paper, we introduce a new cannula system that overcomes these problems by a solid connection of a cannula system to a protective spring. By connecting the injection cannula via waterfilled polyethylene tubing to a Hamilton microsyringe that is placed outside the cage, injections can be made without disturbing the ongoing social interaction. The spring protects the tubing against nibbling and pulling by other animals in the testing cage. The proportions of the system (diameter 3.5 mm, total height of base part and screw cap 9 mm) and its weight (0.8 g.) enable even small animals, like rats, to move freely without noticable hindrance of the cannula system.

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[1] *T.A.P. Roeling, E. Hekman, J. Helmer & J.G. Veening, Physiol. Behav. (1992) in press*

Cannula system.

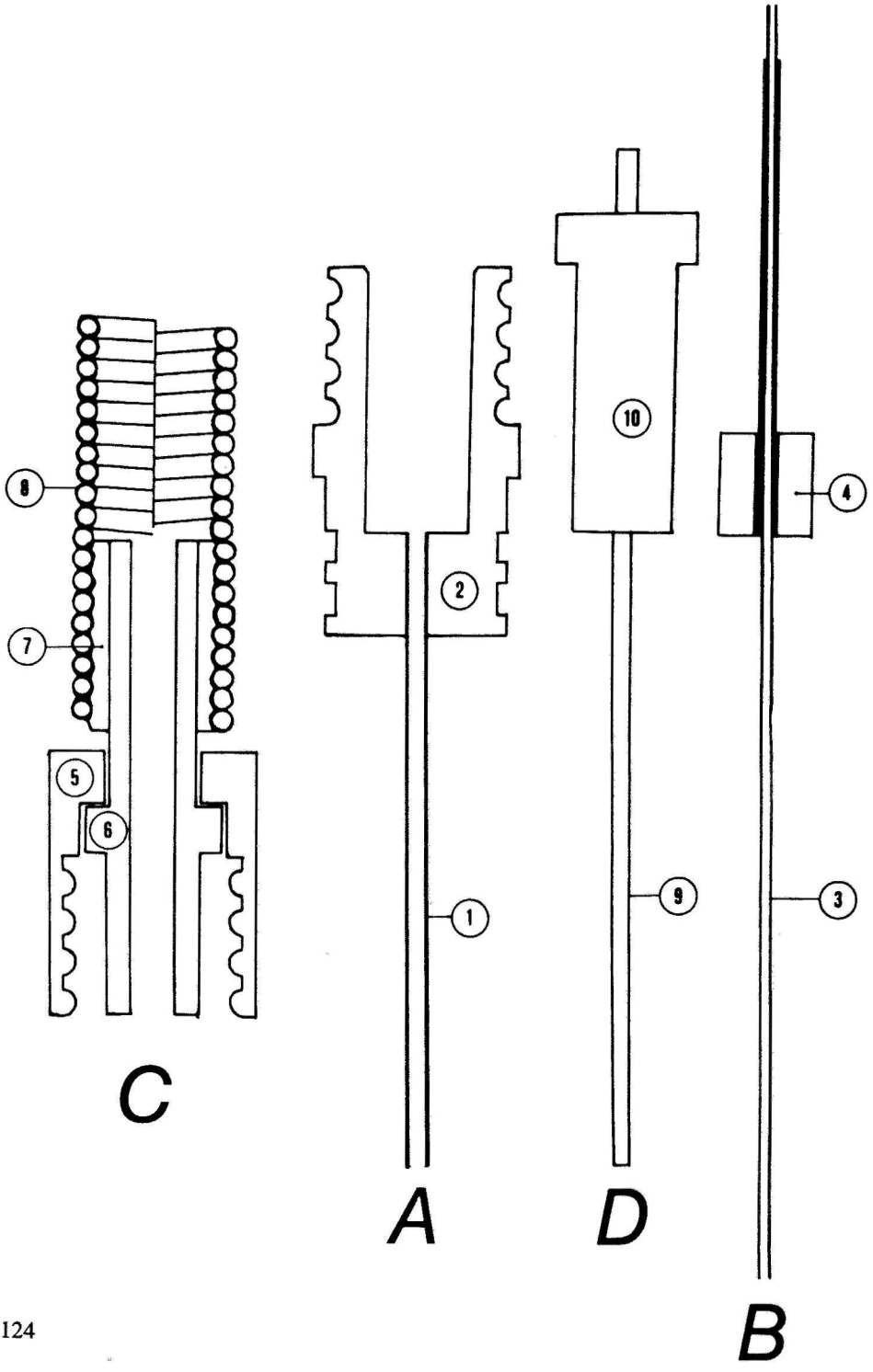
The cannula system consists of three components: 1) a base part with guide cannula (Fig.1A), 2) an injector assembly, consisting of an injection cannula with bush (Fig.1B), 3) a spring assembly consisting of a spring, a threaded ring, a bush, and a screw cap (Fig.1C). Except for the injection cannula and guide cannula, all parts are made by our instrumental service. The guide cannula (o.d. 0.4 mm, i.d. 0.3 mm, Minitubes, Grenoble, France) is fitted into the silvered brass base part. With the aid of a stereotaxic apparatus, the guide cannula can be placed into the brain region of choice. The base part is attached to the skull with dental cement, anchored with two or three stainless steel screws. Grooves on the outer surface of the base part assure steadiness of the attachment.

During the experiment, the injector assembly and the spring assembly are placed into the base part. The length of the injection cannula (o.d. 0.28 mm, i.d. 0.18 mm, Minitubes, Grenoble, France) that is fitted into the silvered brass bush, is adjusted so that it extends one or two (as required) mm beyond the guide cannula. The basepart is closed with a silvered brass screw cap which is connected via a turnable bush and threaded ring to the stainless steel spring. When put together, the injection assembly and the spring assembly are freely rotatable within the base part which is closed with the screw cap. The injection assembly is connected to a Hamilton microsyringe via waterfilled polyethylene tubing. The spring is balanced by a counter weight. This allows the animal to move freely in the cage without tripping over the spring.

Before connecting the animal to the injection device, special care has to be taken that no leakage occurs and that no air bubbles are present in the tubing connecting the Hamilton microsyringe with the injection cannula, except for a small air bubble in a visible part of the tubing, i.e. not within the protective spring and the air bubble separating the water and the injection fluid. Using these precautions, the movement of the visible air bubble during the injection or infusion is an indication that fluid is delivered into the brain. The distance that is passed by the air bubble is a reference for the amount of fluid that is injected. Since the polyethylene tubing we use has an inner diameter of 0.3 mm, movement of the air bubble of 2.8 mm equals the delivery of 0.2 µl of fluid into the brain.

The drug solution is sucked into the injection cannula and secluded from the water in the tubing by a small air bubble (0.1 µl). An extra air bubble (0.1 µl) between the drug solution and the brain tissue prevents leakage of the substance untimely. After replacement of the animal into the testing cage, a period of time (registered time in our experiments 5-20 min) can pass to allow the animal to recover from the handling. In our experiments, drug injections were made at a volume of 0.2 µl in a time period of 30 sec. To make the injection, the plunger of the Hamilton syringe outside the cage is pushed and the drug is delivered into the brain. A small red light outside the cage indicates the time of drug injection for the video recording of the ongoing behaviour. Video recordings have been made up to a period of 30 minutes without any problems with the injection device.

After injection and behavioural recording, the animal is taken out of the experimental cage and disconnected from the injection device. By pressing the Hamilton microsyringe a little further, the immediate occurrence of a symmetrically round droplet indicates that no clogging has occurred.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Between experiments, a dummy, consisting of a steel wire and a bush (delrin), is inserted into the base part (Fig.1D). When the screw cap is fastened, the dummy is tight and cannot rotate anymore. The wire of the dummy does not extend beyond the guide cannula.

The advantages of this new cannula system are clear: microinjections of small (0.2 µl) volumes of neuroactive substances into the brain of freely moving small laboratory animals. By the use of a protective spring, these experiments can be performed in a social environment without disturbance by the experimentor. This system has been used for our experiments to study agonistic behaviour and has proven to work satisfactorily. The present system may be more useful for studies of brain mechanisms of social behaviour than other types of local drug administration, such as microdialysis techniques [1,12] that have been reported thus far, because it is proof against the nibbling, biting, pulling etc. of a conspecific. Another advantage is that it can be re-used. The smaller size and height of the present system as compared to other injection devices, such as the EMIT system [2], allows small animals to move freely in the cage. This cannula system is not only very useful for instantaneous injections and behavioural observations of limited duration, but can also be used for microinfusion during a considerable longer period of time: infusions during a period of 10 minutes have been made without problems (Van Erp et al., pers. comm.).

opposite page:

Figure 1. Parts of the cannula system. A: base part, consisting of 1: guide cannula and 2: base part. B: injection assembly, consisting of 3: injection cannula and 4: bush. C: spring assembly, consisting of 5: cap, 6: bush, 7: threaded ring and

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3.4 BEHAVIOURAL RESPONSES OF BICUCCULLINE METHIODIDE INJECTIONS INTO THE VENTRAL HYPOTHALAMUS OF FREELY MOVING, SOCIALLY INTERACTING RATS[2]

Summary

Several studies involving electrical stimulation of parts of the hypothalamus, have shown, that different parts of the hypothalamus yield different behavioural responses upon stimulation. In order to differentiate between stimulation of neuronal cell bodies and passing fibres, 25 local injections with bicucculline methiodide, a GABA antagonist, (35 ng/ 0.2 µl) were performed in the ventral parts of the hypothalamus of 16 freely moving rats in a social environment. A cannula system was used that allowed injection without interruption of the ongoing social interactions. Digging, gnawing, drinking and attack behaviour were elicited in different animals. A number of injections elicited a combination of two or three different responses. By plotting the behavioural responses of the animals into a detailed hypothalamic atlas, we assessed the hypothalamic distribution of the elicited behavioural responses. Our results are in general agreement with previous electrical stimulation data and show that, in an overlapping pattern, different populations of neurons are involved in the elicitation of digging, gnawing, drinking and attack behaviour. In the hypothalamus, a GABAergic inhibition of neurons involved in the display of these behaviours appears to exist.

Introduction

The hypothalamus is involved in the regulation of a number of behaviours, together with associated autonomic and endocrine responses [8, 29]. By electrical stimulation of hypothalamic sites different behavioural responses can be elicited. It has become clear that each behavioural response that is elicited, has its own preferential location in the hypothalamus, although a number of these behaviourally determined hypothalamic areas show considerable overlap [14, 15, 16]. The neuronal substrate underlying these responses is still largely unknown. Electrical stimulation does not allow the differentiation between activation of passing fibres and neuronal cell bodies. Chemical stimulation has proven to be a useful method to study the involvement of hypothalamic neurons in behaviours that have been elicited by electrical stimulation of the hypothalamus [24, 25, 31].

In this paper we present the results of a study on the elicitation of behaviour after chemical stimulation of the area ventral to the fornix. Electrical stimulation of this area has revealed that different types of behaviour can be elicited, depending on the location of the site that is stimulated, e.g. digging, social grooming, attack and flight [14, 15, 16]. Since

[2] *T.A.P. Roeling, M.R. Kruk, R. Schuurmans & J.G. Veening, submitted for publication.*

used that allows intracerebral injections without disturbing the ongoing social interaction [26]. Bicucculline methiodide (BM), a GABA antagonist, has been used to elicit the behavioural responses. Local injections of BM in the medial hypothalamus have been reported to elicit flight reactions and it has been suggested, that GABA at this level tonically inhibits the display of flight behaviour [2, 4, 27].

Material and methods

Under pentobarbital anaesthesia (Narcovet, Organon, Oss, The Netherlands), 16 male Wistar rats (Central Animal Laboratory, Nijmegen, The Netherlands), weighing 300-400 g, were cannulated, using the coordinates RC -1.9, ML 1.0, DV 8.2 from Bregma [21]. The cannulae were kept in place by dental carboxylate cement and anchored to the skull by stainless steel screws. After cannulation, the animals were solitary housed in macrolon cages in a reversed day-night schedule. They were allowed to recover for at least 1 week, after which they were handled and made familiar with experimental circumstances during 3 sessions in the following week. A familiarization session consisted of holding the animal in the experimenter's hand during 3 minutes, connecting the animal to the injection system without injection cannula and placing the animal in the experimental cage for 15 minutes. In the third session, a male conspecific (150- 200 g) was placed in the experimental cage.

In experimental sessions, both experimental and partner animals were first allowed to explore the cage (50x50x75 cm) for at least 10 minutes, after which the injection cannula was inserted through the guide cannula, extending 1 mm beyond the tip of the guide cannula. The injection cannula was connected via waterfilled polyethylene tubing to a Hamilton microsyringe. The tubing was protected by a turnable stainless steel spring, attached to the skull [26]. The spring was balanced by a counterweight. Five minutes later video recordings started. Preinjection recording time lasted 6- 10 minutes. The injection with bicucculine methiodide (BM) (35 ng/ 0.2 µl saline) was made over a period of 30 seconds. Postinjection recording time was 9-15 minutes. Except for the animals that displayed attack behaviour, all animals received a second BM injection, 1 mm deeper than the previous injection 48 hours later.

The video recordings were analysed using behavioural encoding equipment (Soliprot, Biology Department. Leiden University, The Netherlands) and specially designed software. In the analysis, digging, gnawing, drinking, attack, social behaviour (social grooming, partner sniffing), exploratory behaviour (locomotion, rearing and sniffing) and selfdirected behaviour (grooming, sitting) were considered as separate behavioural elements. Gnawing, drinking, digging and attack were further investigated for possible effects of hypothalamic stimulation. All durations of behaviours recorded in the pre- or in the post-injection period were calculated as percentage of the recorded time (%TT). We considered an injection effective in eliciting a particular response if the time spent on a specific response exceeded the mean time plus standard error of the mean of the whole group of injections as measured during preinjection periods as well as exceeded the duration of time spent on this behaviour as performed by the individual animal in the preinjection period. In the animals, that displayed attack behaviour, saline injections were made 48 hours after the BM injection, using the same paradigm. A second BM injection was made 48 hours to

verify this behavioural response.

Upon completion of the experiments, the animals were perfused with 4% paraformaldehyde/ 0.05% glutaraldehyde in 0.05 M. phosphate buffer (pH 7.4). The brains were stored overnight in phosphate-buffered 10% sucrose solution. Frozen sections (40 μm.) were stained with cresylviolet. The injection sites were plotted in a new cytoarchitectonic atlas of the hypothalamus of the rat [5,6].

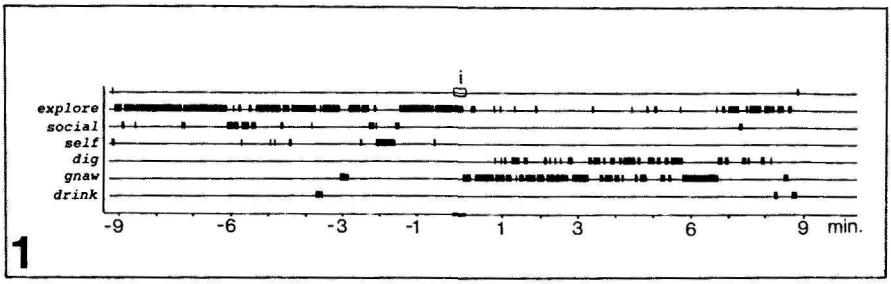
Results

During the preinjection period, the animals performed exploratory behaviour (mean ± S.E.M. 38.7 ± 4.3 %TT), selfdirected behaviour (mean ± S.E.M. 42.3 ± 5.3 %TT) and social behaviour (mean ± S.E.M. 10.0 ± 1.7 %TT). The types of behaviour of interest in the present study were only sparsely observed in this period: digging: 1.0 ± 0.4 %TT; gnawing: 7.4 ± 1.8 %TT; attack: 0.2 ± 0.1 %TT; drinking: 0.4 ± 0.3 %TT (mean ± S.E.M.).

Figure 1 shows an example of an injection, where digging and gnawing is induced by injection of BM. The onset of these types of behaviour was usually very quick and appeared within 10 seconds after cessation of the injection. The effect lasted for about 8 minutes. During this period, only very few other behavioural activities were noticed. After 8 minutes, the animal returned to the behaviour, in which it was engaged in before the injection. We were not always able to distinguish between gnawing and feeding, due to the low level of illumination of the experimental cage, which did not always allow detection of swallowing movements. For the gnawing response, usually scattered food pellets were used instead of the woodblocks. In the home cages, woodblocks were frequently used for gnawing.

Figure 2 shows an example of an injection resulting in attack behaviour, subsequently followed by drinking behaviour. The attack behaviour usually lasted for only a brief period (2 minutes). The attacks, however, were usually fierce and included various forms of attacks, like clinch fights, bite attacks and kick attacks [10]. Interestingly, in this series, no sideways threat has been noticed. Attack was elicited in five injection sites. In three of these sites, a second identical BM injection 96 hours later elicited the same response; one injection did not result in attack behaviour, but only high amounts of locomotion. One animal did not receive a second injection, due to technical problems. Saline injections in between the BM injections in these animals never resulted in any behavioural change as compared to the preinjection period.

The behavioural aspects of the present study have been summarized in figure 3. The mean results of all preinjection periods (n=29) have been indicated as bars ± S.E.M., and show that digging, gnawing, drinking and attack behaviour occurred only rarely before the injection of BM. Using the criteria mentioned in the materials and methods section, 7 injection sites were considered as having elicited a digging response; 8 injection sites elicited gnawing; 5 injection sites elicited attack and 7 injection sites elicited drinking. The effects of these injections are indicated in figure 3 as single dots.



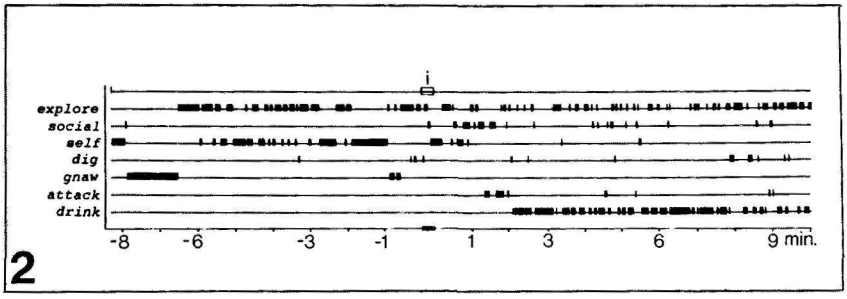


Figure 1. *Event plot of the behaviour of rat R251-15 before and after injection of bicucculline methiodide, revealing the elicitation of digging and gnawing. i = time of injection.*

Figure 2. *Event plot of the behaviour of rat R300-14 before and after injection of bicucculline methiodide, revealing the elicitation of attack behaviour and subsequent drinking. i = time of injection.*

The location of the injection sites is depicted in figure 4. Positive sites for digging, gnawing, attack and drinking are indicated with different symbols. If one injection elicited more than one single behavioural response, the different symbols are superimposed on top of each other. In general, the injection sites where gnawing was elicited were situated most lateral in the hypothalamus. Sites where digging was elicited were situated medial to the area where gnawing was elicited. Although the sites where attack was elicited did not cluster evidently, they appeared to be situated medial to the sites where gnawing and digging were elicited, viz. lateral to the ventromedial hypothalamic nucleus (VMH). Drinking was elicited in injection sites that appeared to be placed ventral to the fornix and dorsolateral to the attack sites.

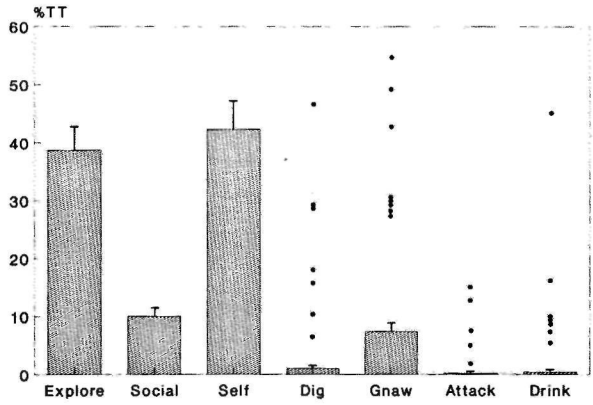


Figure 3. *Bar plot of the total time spent on different types of behaviour in preinjection time as percentage of the total observed time (Mean ± S.E.M.). Behavioural responses of different animals, that were regarded to be an effect of the injection of bicucculline methiodide, are indicated as single dots.*

Discussion

The use of electrical stimulation has long been used for the study on hypothalamically elicited behavioural responses [8, 9, 10, 11, 14, 15, 16]. The results of the present study clearly show that within the hypothalamus, neuronal cell bodies are involved in the elicitation of a number of these behaviours, viz. digging, gnawing, drinking and attack. At the level of the hypothalamus, GABA may be involved. Local injection of GABA antagonists in the hypothalamus has been performed earlier to elicit flight behaviour [2, 4, 27]. In our experiments, in only two injection sites flight behaviour was elicited. These injection sites were positioned in the medial hypothalamic area dorsal to the ventromedial hypothalamic nucleus. Although no detailed analysis has been made on the localization of neurons that are involved in flight behaviour, the medial location within the hypothalamus, as it was found in the present study, is in agreement with earlier reports on this behaviour elicited by electrical stimulation and GABA antagonists [2, 4, 15].

The present study is intended to show the involvement of neuronal cell bodies in hypothalamic responses and the possible role of GABA and involves insufficient numbers of injection sites to allow a full description of the spatial organization of the response areas. However, the sites where drinking, gnawing, digging and attack were elicited, are in agreement with previous reports using electrical stimulation.

Attack behaviour was elicited in five injection sites. Saline injections at the same site in between the bicucculline methiodide (BM) injection sessions, induced no attack behaviour, indicating that the behaviour was elicited by BM. In a total of eight recordings of attack behaviour induced by BM injection no sideways threat was noticed. This is in agreement with earlier observations on electrically elicited aggressive behaviour [9, 10, 13]. The

injection sites were all positioned lateral to the ventromedial hypothalamic nucleus. It has been reported before, that electrical stimulation of this part of the hypothalamus elicits attack behaviour [9, 11, 12, 13, 16]. Although the number of injections eliciting attack behaviour in the present study was small, it is likely that neurons in that specific part of the hypothalamus are involved in the regulation of attack behaviour.

The role of the hypothalamus in the regulation of digging has been investigated only sparsely [14, 32]. The sites where digging was elicited by electrical stimulation were largely similar to our sites, although our sites appeared to be situated a little more ventromedial than the sites where electrical stimulation elicits digging [14, 32]. The lack of knowledge about the exact location of the neuronal cell bodies that were activated, does not permit to clarify this discrepancy.

The position of sites where gnawing was induced are in agreement with earlier reports on the elicitation of feeding by electrical stimulation of the lateral hypothalamus [1, 3, 8, 18, 20, 23]. The involvement of neuronal cell bodies in the lateral hypothalamus in the control of feeding and drinking, has already been suggested by the effects of local destruction of lateral hypothalamic cell bodies using kainic acid, resulting in aphagia and adipsia [7, 28, 33]. Although in our studies no certainty was achieved whether the animals were eating or only gnawing, the choice of the food pellets by the animals instead of the woodblocks suggests that the behaviour that was elicited is related to feeding behaviour. The finding that gnawing as a result of electrical stimulation of the lateral hypothalamic area occurred only infrequently when compared to feeding and drinking, supports this view [20].

Drinking behaviour is known to be mediated by the subfornical organ, which projects to the medial preoptic area and hypothalamic paraventricular area [17, 29, 30]. Electrical stimulation of the lateral hypothalamic area has been reported to induce drinking as well [8, 19, 20, 23]. In the studies on feeding and drinking elicited by electrical stimulation of the lateral hypothalamic area, no clear differentiation has been made in the location of sites where specifically feeding or drinking can be elicited [3, 18, 20, 22, 23]. Our results suggest that at the level of the hypothalamus different populations of neurons are involved in these behaviours, although there may be overlap in the spatial organization.

In the present study, there were several sites where a single injection resulted in the elicitation of two or even three different behaviours. Injection sites eliciting two behaviours were frequently found in between areas where only one response could be induced. An explanation for the elicitation of two behaviours by a single injection could be the spread of the injection fluid which may reach two different populations of neurons. This is in agreement with earlier findings that the hypothalamic areas where specific behaviours can be elicited by electrical stimulation show considerable overlap, while the centres of the different behavioural areas are well separated [14, 15, 16]. The use of total duration for our description of behaviour may have obscured a possible differentiation in the occurrence of different behaviours over time after injection. Especially in experiments where both attack and drinking were induced after a single injection, attack behaviour was displayed before drinking (Fig.2). Digging and gnawing, on the other hand, did not show a difference in latency (Fig.1). More experiments and more extensive behavioural studies are therefore necessary to elucidate the cause and the distribution over time of successively induced behavioural responses.

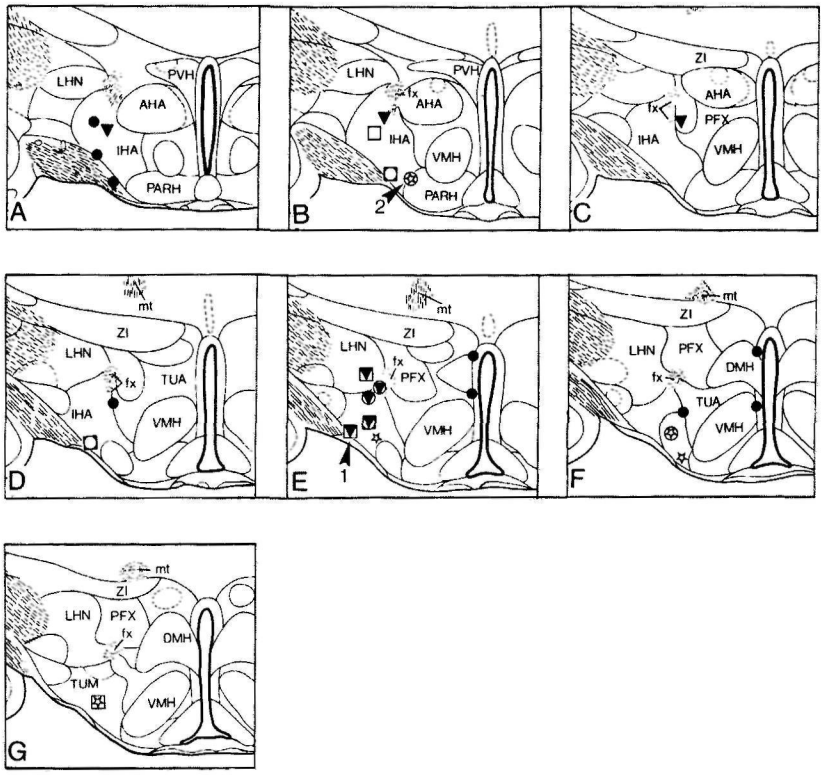


Figure 4. Distribution of injection sites on frontal planes from rostral (A) to caudal (G) from the atlas of Geeraedts et al. [5,6]. *Sections are 150 µm apart. Indication of the symbols: digging:□;gnawing:▼;drinking:○;attack:✲;no/ other responses:●. Abbreviations: AHA: anterior hypothalamic area; ci: capsula interna; DMH: dorsomedial hypothalamic nucleus; fx: fornix; IHA: intermediate hypothalamic area; LHN: lateral hypothalamic nucleus; mt: mammillothalamic tract; ot: optic tract; PARH: para- arcuate hypothalamic nucleus; PFX: perifornical nucleus; PVH: paraventricular nucleus; TUA: area of the tuber cinereum; TUM: medial tuberal nucleus; VMH: ventromedial hypothalamic nucleus; ZI: zona incerta.*

In conclusion, it can be stated that hypothalamic neurons are involved in the elicitation of digging, gnawing, drinking and attack behaviour and that there appears to be a spatial organization of these behaviours within the hypothalamus, although there may be considerable overlap. At the level of the hypothalamus, a GABAergic inhibition for the display of these behaviours may be involved.

Acknowledgements

The authors wish to acknowledge Eva Maria Braun for assistance in the experiments, Marion Vermelis for histological assistance, and Fred Roeling for the development of the software used for the analysis of the behaviour.

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3.5 EFFERENT CONNECTIONS OF THE HYPOTHALAMIC "AGGRESSION AREA" IN THE RAT[1]

Abstract.

The efferent connections of the hypothalamic area where attack behaviour can be elicited by electrical stimulation, were studied using iontophoretic injections of *Phaseolus vulgaris* leucoagglutinin (PHA-L). The specificity of projections from the hypothalamic "attack area" was investigated by comparison with efferents of hypothalamic sites outside the "attack area". The hypothalamic "attack area" consists of the intermediate hypothalamic area and the ventrolateral pole of the ventromedial hypothalamic nucleus.

Fibres from the hypothalamic attack area, as well as fibres from several other hypothalamic sites, form diffuse fibre "streams" running rostrally or caudally. The presence of many varicosities en passant suggests that these fibres are capable of influencing many brain sites along their way.

Projection areas were found throughout the brain. Comparing "attack area" efferents and controls, many overlapping projection areas were found. Preferential efferents from the largest part of the "attack area", *i.e.* the intermediate hypothalamic area, were observed projecting to the mediodorsal and parataenial thalamic nuclei. Within the septum, a spatial organization of hypothalamic afferents was found. Fibres from the "attack area" formed specialized "pericellular baskets" in the dorsolateral aspect of the intermediate part of the lateral septal nucleus. Fibres from other hypothalamic sites were found in other septal areas and did not form these septal baskets. Within the mesencephalic central gray, fibres from the "attack area" were found specifically in the dorsal part and in the dorsal aspect of the lateral part of the central gray.

Physiological and pharmacological studies have shown that several brain areas are involved in different aspects of aggressive behaviour. Some of these areas, as for instance the dorsomedial thalamic nucleus, septum and central gray, are innervated by efferents from the hypothalamic "attack area", whereas other areas, like ventral premammillary nucleus and ventral tegmental area, do not receive such fibres.

It is concluded from the present findings that a number of brain areas known to be involved in agonistic behaviour, receive specifically information from the hypothalamic "attack area" through diffusely arranged varicose fibres. The knowledge of the function of each connection in the regulation of specific types of behaviour remains to be investigated in the further study of brain mechanisms of behaviour.

[1]*T.A.P. Roeling, J.G. Veening, M.R. Kruk, J.P.W. Peters, M.E.J. Vermelis & R. Nieuwenhuys.*

Introduction

The hypothalamus plays an important role in the integration of behaviour and associated autonomic and endocrine responses [66]. By using electrical stimulation of parts of the hypothalamus, it has been demonstrated that many different types of behaviour can be elicited, like locomotion, feeding, drinking, aggression, digging and grooming [28,31,36,37,38]. The site of stimulation appears to be an important determinant of the type of behaviour that is elicited, although sites from which one type of behaviour can be elicited appear to overlap with sites from where other types of behaviour can be elicited [36,37,38]. In addition, these behavioural maps may not agree with the borders of cytoarchitectonically defined hypothalamic nuclei and areas.

Since different types of behaviour require different levels of autonomic activity in a variety of organs, different types of behaviour require the activation of different brain areas [56]. Therefore, a hypothalamic area from where one type of behaviour can be elicited should have other efferent connections than hypothalamic areas from where another type of behaviour can be elicited.

In the present paper we present the efferent connections that are possibly involved in the regulation of hypothalamically elicited attack behaviour. The specificity of these efferents will be investigated by comparing them with the efferent connections of hypothalamic areas outside the hypothalamic "attack area". Attack behaviour has been elicited by electrical stimulation of a part of the hypothalamus that is located ventral to the fornix and includes the intermediate hypothalamic area (IHA) and the ventrolateral pole of the ventromedial hypothalamic nucleus (VMH) [31,32,33,34,35,38,69]. By local injections in the same hypothalamic area with the GABA antagonist bicuculline methiodide, attack behaviour was elicited [57]. This suggests that hypothalamic neuronal cell bodies are involved [57]. In the present report, we delineated the HAA using a large number of positive electrode placements of previous studies [32,34,38]. Subsequently, the efferent connections of hypothalamic areas within and outside the HAA were investigated and compared using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) [24]. The destination of the efferent connections of the HAA is compared with brain areas that have been reported in the literature to be involved in agonistic behaviour [2,5,12,15,20,30,44,61]. Within these brain areas, the amygdalo-hypothalamo-central gray axis seems to play an important role, but other brain areas, like mediodorsal thalamic nucleus, septum, ventral tegmentum and prefrontal cortex have also been associated with agonistic behaviour [2,4,5,15,42].

Materials and methods

Fourty-six male Wistar rats (CDL, Nijmegen, The Netherlands) were used. Under pentobarbital anaesthesia (Narcovet, Organon, 1ml/kg) the animals were placed in a stereotactic apparatus. Iontophoretic deliveries (3-5 μA, 10 min.) of *Phaseolus vulgaris* leucoagglutinin (PHA-L, Vector, 5% in phosphate buffer) were made aimed at the coordinates AP -1.8 mm, ML 1.0 mm, DV -8.6 mm from Bregma [55]. After a survival time of 7-14 days, the animals were deeply anaesthetized with pentobarbital (1.5 ml/kg) and transcardially perfused with 100 ml saline and subsequently with 300 ml fixative (2.5 % paraformaldehyde, 0.05 % glutaraldehyde in 0.1 M phosphate-buffered (PB) saline, pH 7.6). Brains were taken out of the skull and stored overnight in 20 % sucrose solution (PB,

pH 7.6). Transverse 40 µm frozen sections were cut and 2 out of 5 sections were stained for PHA-L detection, one of which was counterstained with Nissl staining. Sections were rinsed for 2 hrs with Tris-buffered saline (TBS). Sections were incubated at room temperature overnight in TBS containing biotinilated anti- Phaseolus antibody (Vector laboratories). After rinsing with TBS (3x) sections were incubated with ABC fluid (Vector laboratories) for 1 hr and stained with diaminobenzidine (DAB), using ammonium nickel sulphate for staining intensification. Sections were coverslipped with Entellan.

Fibre patterns were drawn on outlines of sections that were taken from the atlas of Paxinos and Watson [55]. The hypothalamic and preoptic regions were redrawn using the detailed atlas of Geeraedts et al. [22,23], from which part of the nomenclature is also taken.

Results.

Delineation of the hypothalamic "attack area"

Positive electrode placements from previous experiments [32,34,38] were replotted on a detailed cytoarchitectonic atlas of the hypothalamus [22,23] (Fig.1). Most electrode placements were situated in the intermediate hypothalamic area (IHA), with the most rostral site at the level of the hypothalamic paraventricular nucleus (PVH) (Fig.1A). Caudal to the PVH the electrode placements are situated more medially and a small number is found in the ventrolateral pole of the ventromedial hypothalamic nucleus (VMH) (Fig.1D-F). The hypothalamic "attack area" (HAA), as delineated and defined in this study, is indicated by stippling in figure 2. It consists largely of the intermediate hypothalamic area (IHA), but includes parts of the ventrolateral pole of the ventromedial hypothalamic nucleus (VMH) as well.

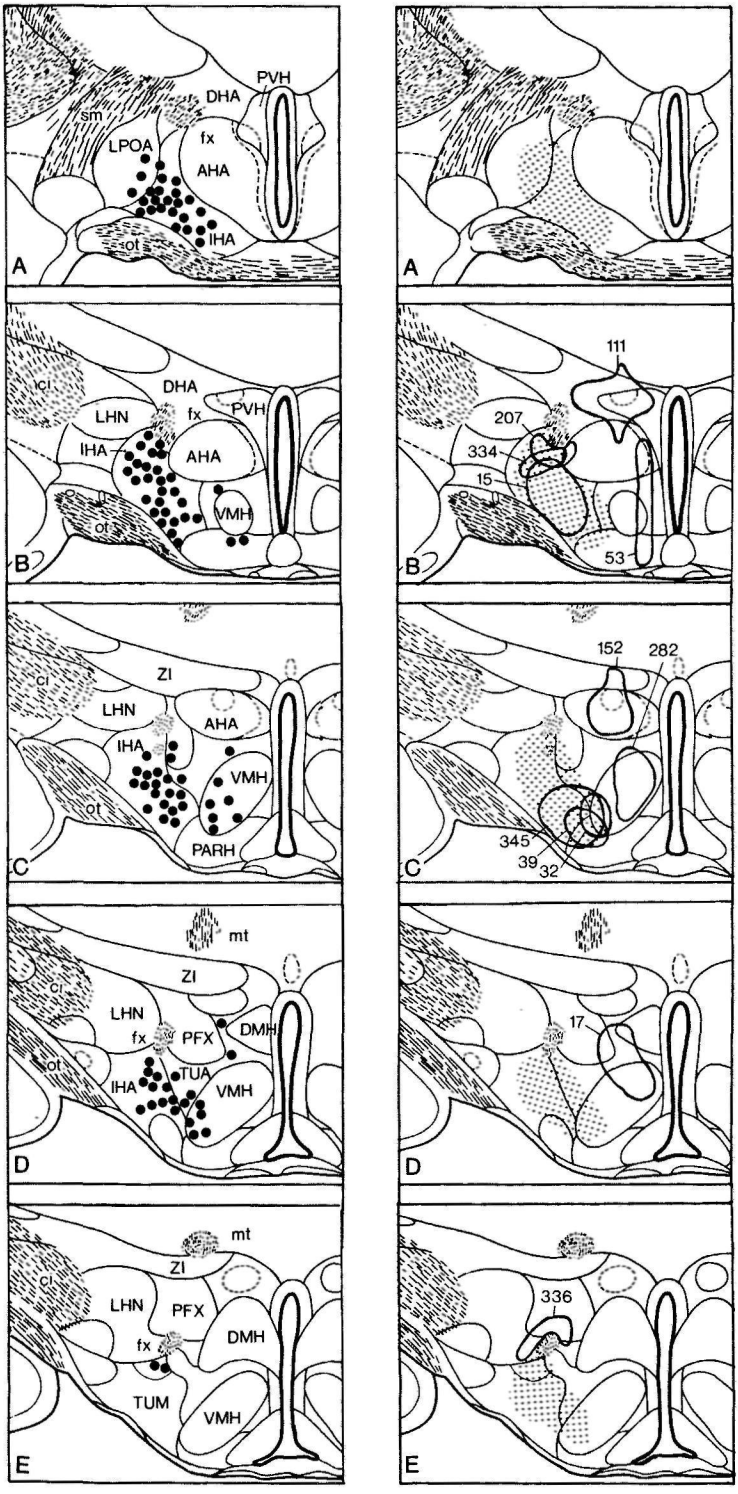
PHA-L injection sites of the HAA and controls.

An example of a PHA-L injection in the HAA has been given in Fig.3 (Rat R345). Neuronal cell bodies are labelled including their first and second order dendrites. From the injection site, labelled fibres spread in various directions. Many of these fibres contain varicosities "en passant", indicating possible locations of synapses along the course of these fibres rostrally or caudally [75]. Therefore, a description of the pattern of these efferent fibre "streams" was considered useful.

next page:

Figure 1. Replotting of electrode placements where attack behaviour has been elicited in the hypothalamus with electrical stimulation [32,39,38]. Drawings from rostral (A) to caudal (E), taken from Geeraedts et al. [22,23]. For abbreviations: see list of abbreviations.

Figure 2. Injection sites, that have been used in the present study. The largest extension of the injection sites have been plotted. The hypothalamic "attack area" has been indicated with stippling. For abbreviations: see list of abbreviations.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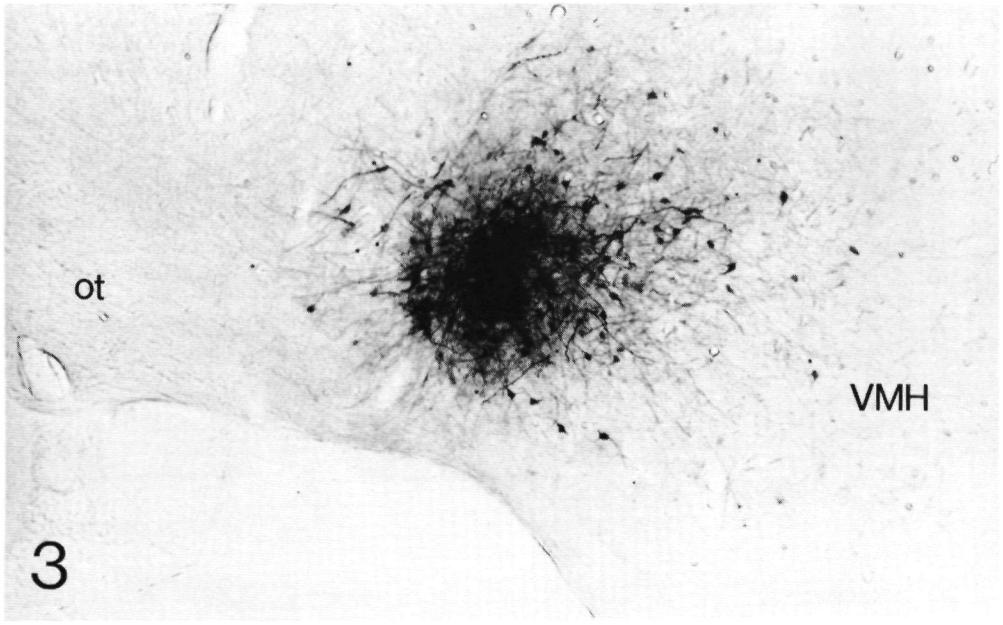


Figure 3. *Photomicrograph of a PHA-L injection into the intermediate hypothalamic area (rat R345). Scale bar: 100 μm.*

From the total number of 46 injection sites, 12 series have been used for the present study. These injection sites are indicated in figure 2. Six injections were placed in the HAA, two of which in the dorsal part of the IHA (R207, R334), two in the ventral part of the IHA (R15, R345) and two small injections in the ventrolateral pole of the VMH (R32, R39). Six injections were placed outside the HAA, one of which in the lateral hypothalamic area (LHA)/ perifornical nucleus (PFX) (R336), one in the hypothalamic paraventricular nucleus (PVH)/ dorsal hypothalamic area (DHA) (R111), one in the anterior hypothalamic area (AHA) (R152) and three dorsomedial in and dorsal to the hypothalamic ventromedial nucleus (VMH) (R53, R282, R17).

Three series will be discussed in some detail. Labelled fibres from an injection site in the HAA are shown in figure 6 (R345). The injection is placed in the intermediate hypothalamic area (IHA) ventral to the fornix at the level of the largest extension of the ventromedial hypothalamic nucleus (VMH). A small number of VMH neurons are labelled as well (Fig. 2). The injection site is shown in figure 3. Labelled fibres from a control injection site including the dorsal part of the VMH and parts of the dorsomedial hypothalamic nucleus (DMH) are shown in figure 7 (R17). Labelled fibres from a control injection in the LHA/ PFX are shown in figure 8 (R336).

List of abbreviations.

7n	facial nerve
A5	noradrenergic A5 area
AA	amygdaloid area
ac	anterior commissure
Acb	nucleus accumbens
ACo	anterior cortical nucleus
AHA	anterior hypothalamic area
AHiA	amygdalohippocampal area
Arc	hypothalamic arcuate nucleus
Bar	Barrington's nucleus
BLA	basolateral amygdaloid nucleus, anterior
BLP	basolateral amygdaloid nucleus posterior
BMA	basomedial amygdaloid nucleus anterior
BMP	basomedial amygdaloid nucleus posterior
BST	bed nucleus stria terminalis
BSTIA	bed nucleus stria terminalis intra- hippocampalis
C1/A1	medullary C1/A1 area
CA1	hippocampal CA1 area
CeA	central amygdaloid nucleus
CG	mesencephalic central gray
CGPn	central gray, pons
CI	colliculus inferior
Cl	claustrum
CM	centromedial thalamic nucleus
CnF	cuneiform nucleus
cp	cerebral peduncle
CPu	caudatoputamen
CS	colliculus superior
CTF	central tegmental field
dac	decussation anterior commissure
DBB	diagonal band Broca
DEn	dorsal entorhinal nucleus
DMH	dorsomedial hypothalamic nucleus
DMV	dorsal motor nucleus vagal nerve
DPM	dorsal premammillary nucleus
DR	dorsal raphe nucleus
fr	fasciculus retroflexus
fx	fornix
GP	globus pallidus
ic	internal capsule
IHA	intermediate hypothalamic area
LaA	lateral amygdaloid nucleus
LC	locus coeruleus
LHA	lateral hypothalamic area
LHb	lateral habenular nucleus
LPB	lateral parabrachial nucleus
LPOA	lateral preoptic area
LRt	lateral reticular nucleus
LSD	lateral septal nucleus, dorsal part
LSI	lateral septal nucleus, intermediate part
LSO	lateral superior olive nucleus
LSV	lateral septal nucleus ventral part
MD	mediodorsal thalamic nucleus
MdD	dorsal medullary reticular field
MdV	ventral medullary reticular field
ME	median eminence
MeA	medial amygdaloid nucleus
MG	medial geniculate nucleus
ml	medial lemniscus
mlf	medial longitudinal fasciculus
mt	mammillothalamic tract
MnPO	median preoptic nucleus
MnR	median raphe nucleus
MPB	medial parabrachial nucleus
MPOA	medial preoptic area
MS	medial septal nucleus
NTS	nucleus solitary tract
ot	optic tract
OT	nucleus optic tract
OTU	olfactory tubercle
ox	optic chiasma
OVLT	organum vasculosum of the lamina terminalis
PeAV	anterior periventricicular nucleus
PHA	posterior hypothalamic area
PIL	posterior intralaminar thalamic nucleus
Pir	piriform cortex
PMCo	cortical amygdaloid nucleus posteromedial part
PMR	paramedian raphe nucleus
POM	medial preoptic nucleus
POMA	magnocellular preoptic nucleus
PP	peripeduncular nucleus
PPTg	nucleus tegmentalis pedunculopontinus
PrC	nucleus precommissuralis
Pt	parataenial thalamic nucleus
PVH	paraventricular hypothalamic nucleus
PVT	paraventricular thalamic nucleus
py	pyramidal tract
Re	thalamic nucleus reuniens
Rh	thalamic rhomboid nucleus
RMg	nucleus raphe magnus
RPO	rostral periolivary region
rs	rubrospinal tract
Rt	nucleus rotundus thalami
SCh	suprachiasmatic nucleus
scp	superior cerebellar peduncle
SIC	substantia innominata, pars subcommissuralis
SIL	substantia innominata, pars sublenticularis
sm	stria medullaris
SNC	substantia nigra, pars compacta
SNR	substantia nigra, pars reticulata
SON	supraoptic nucleus
st	stria terminalis
SUT	subthalamic nucleus
TUM	medial tuberal nucleus
VL	ventrolateral thalamic nucleus
VLM	ventrolateral medulla
VLTg	ventrolateral tegmental area
VMH	ventromedial hypothalamic nucleus
VPM	ventral premammillary nucleus
VTA	ventral tegmental area
VTg	ventral tegmental nucleus
ZI	zona incerta

Table1

		LSI	LSV	LSD	DBB	MS	SIC	SIL	BST	st	AA	MeA	CeA	LaA	BSTIA	AHiA	CAi	LHb	PVT	sm	MD	Rh	Re	CL	CM	Pt	LPOA	MPOA	LHA
IHA	15	xxx	xx	xx	x	x	xx	x	xxx	xxx	x	xx	x	o	xx	x	o	xx	xxx	xx	xxx	xx	xxx	o	xx	xxx	xxx	xxx	xx
	345	xxx	x	o	xx	xx	xxx	xx	xxx	xx	xx	x	xx	xx	xxx	xxx	xxx	xx	xxx	x	xxx	xx	xx	o	x	xx	xx	xxx	x
	334	xxx	xx	x	xx	xxx	x	xx	xx	xx	x	x	o	o	x	o	x	x	xx	x	xxx	x	x	o	xx	xx	xxx	x	x
	207	xxx	x	x	x	x	x	xx	xxx	xx	xx	xx	x	o	xxx	xx	xx	xx	xx	x	xxx	xx	xx	x	xx	xxx	xxx	xx	x
VMHvl	39	xx	x	o	o	o	x	x	x	x	x	x	xx	xx	xx	o	x	x	xx	xx	xx	o	o	o	x	x	xx	xx	xx
	32	xx	x	o	o	x	xx	x	xx	xx	x	x	x	x	xx	xx	o	xx	xx	x	x	x	o	o	x	x	x	xxx	x
VMH	53	xxx	xx	x	xx	x	xx	xxx	xxx	xx	xxx	xx	xx	xxx	xx	x	xx	xx	xxx	x	xx	xx	xx	x	xx	x	xx	xxx	xx
	282	xx	x	x	x	x	x	x	xx	x	xxx	xx	xx	xx	xx	x	o	x	xx	o	x	x	xx	x	x	o	x	xx	x
VMH/DMH	17	xxx	x	x	x	x	xxx	xxx	xxx	x	xxx	xx	xx	xx	xx	x	o	x	xx	x	xx	xx	o	o	xx	x	xx	xxx	x
PVH/DHA	111	xx	xxx	x	x	o	xx	xx	xxx	xx	xxx	xxx	xx	o	xx	x	xx	xx	xxx	x	xx	xx	xx	xx	xx	x	xxx	xxx	xx
AHA	152	xxx	xx	x	xx	x	x	x	xx	x	xx	x	x	o	xx	x	xx	xx	xxx	x	xx	xx	xxx	x	xx	x	xx	x	xx
LHA/PFX	336	xx	x	xxx	x	x	xxx	x	xx	xx	o	o	o	o	o	o	o	x	x	x	x	x	o	xx	x	o	x	xx	xx

		VMH	DMH	ZI	Arc	ME	VPM	DPM	CG	VTA	CTF	PrC	DR	MnR	VITg	PPTg	CnF	LPB	MPB	LC	Bar	CGPn	A5	RMg	NTS	VLM
IHA	15	xxx	xx	xx	x	o	x	xxx	xxx	x	xx	xxx	xx	xx	x	o	xx	x	o	o	x	x	x	x	o	o
	345	xx	x	x	x	o	x	xx	xxx	xx	xxx	xx	xx	xx	x	xx	xx	xx	xx	xx	x	xx	x	xx	xx	xx
	334	xx	x	o	x	o	x	xxx	xx	x	x	xx	x	xx	x	o	xxx	o	o	o	x	x	o	x	o	o
	207	xx	o	x	o	o	x	xxx	xxx	x	x	xxx	x	x	o	x	xx	x	x	x	x	x	o	o	o	o
VMHvl	39	xx	x	o	xx	x	x	x	xxx	o	xx	x	x	o	x	x	xxx	x	xx	x	o	x	xx	x	o	o
	32	xx	x	xx	xx	x	xx	x	xx	o	xx	xx	o	o	x	x	xx	o	x	x	o	x	xx	x	o	x
VMH	53	xxx	xx	xx	xx	x	o	xxx	xxx	xx	xxx	xx	x	xx	xx	xx	xx	xx	x	xx	o	xx	xx	x	x	x
	282	xxx	x	xx	x	o	x	xxx	xxx	x	xxx	xx	o	x	x	x	xx	x	x	x	o	x	x	x	x	x
VMH/DMH	17	xxx	xx	xxx	xx	x	x	xx	xxx	x	xxx	xxx	x	x	o	xx	xx	x	x	xx	xx	x	x	o	x	xx
PVH/DHA	111	x	xxx	xx	xxx	xx	xx	xx	xxx	xxx	xxx	xxx	xx	xx	x	xx	xx	xxx	xx	xxx	xx	xxx	xxx	xxx	xxx	xxx
AHA	152	xx	xx	xx	x	x	x	xxx	xxx	x	x	xx	x	xx	x	xx	xx	x	o	x	o	xx	x	x	x	x
LHA/PFX	336	o	x	x	x	o	o	x	xxx	xxx	xxx	x	xx	xxx	x	o	xx	xx	xx	xx	xxx	xx	x	xx	xx	x

Table 1.Semiquantitative indication of the innervation of various brain areas after PHA-L injections into the hypothalamic "attack" area and control injections. O: no innervation; x: sparse innervation; xx: moderate innervation; xxx: large innervation. For abbreviations: see list of abbreviations.

In Table 1, a semiquantitative listing is presented of the number of labelled fibres in a number of brain areas.

In the description of the efferents, only the ipsilateral side of the brain will be described. Fibres in contralateral brain areas are found in all series as well, but always in smaller numbers as compared to the ipsilateral side. In the localization of the fibres on both sides no marked differences have been found.

Efferent fibre patterns of the HAA and control sites.

Efferents from the hypothalamic areas that have been studied, tend to cluster in fibre streams coursing rostrally or caudally, although the pattern is very diffuse. In Fig.4 the "flow patterns" are indicated for the fibres leaving the IHA (Fig.4B), the VMH/ DMH injection (Fig.4C) and the LHA/ PFX (Fig.4D). Figure 5 shows in more detail the efferent fibre "streams" of the IHA injection (R345).

Ascending fibre streams.

Fibres running rostrally from the injection sites form three separate fibre "streams": a dorsal, a lateral and a ventral stream.

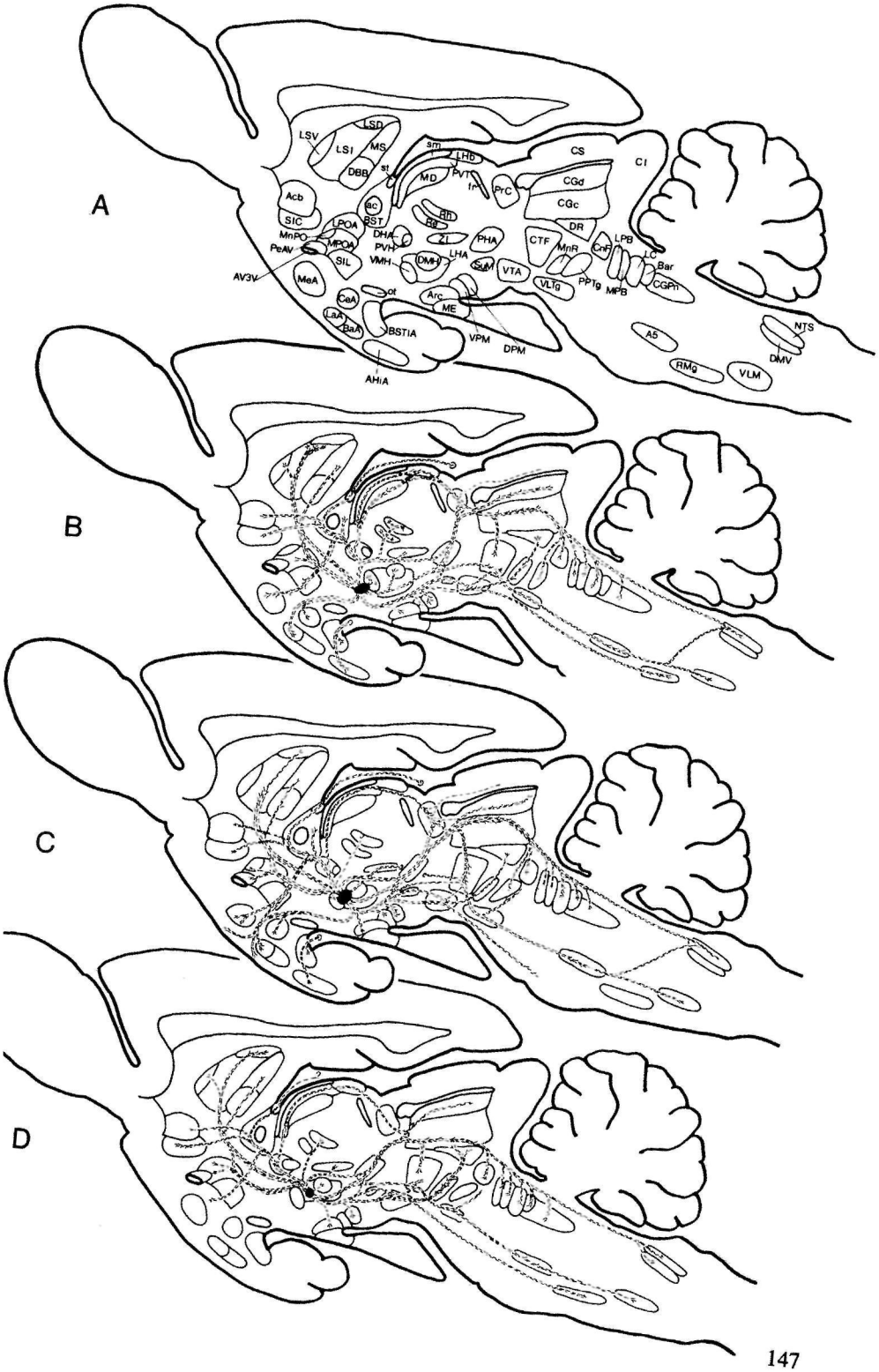
The dorsal ascending fibre stream.

A moderate number of fibres leaving the injection site turns dorsally and runs within the dorsal ascending fibre stream (stream 1, figs.4,5) to the dorsal hypothalamic area and further rostrally to the bed nucleus of the stria terminalis (BST). Within **the bed nucleus of the stria terminalis** (BST) a considerable number of varicose fibres and terminals is found in all series that have been examined (Table 1, Figs.4, 5, 6-8B).

Within the BST, the stream divides into three smaller streams entering either the stria terminalis (st) and the substantia innominata (Figs.4,5). The substantia innominata is in the present study subdivided into a subcommissural (SIC) and a sublenticular (SIL) part, according to Geeraedts et al. [22]. To some extent, this differentiation corresponds to the subdivision in a dorsal and a ventral part as used by Grove [25].A large number of varicose IHA fibres and terminals are found in the **substantia innominata, pars subcommissuralis** (SIC) (Figs.6A,B). The SIC is more extensively innervated by fibres from the ventral part of the intermediate hypothalamic area (IHA) (R345/ R15) than by fibres from the dorsal IHA (R207/ R334) (Table 1). The ventromedial hypothalamic nucleus (VMH) projects moderately to the SIC (Figs 7A,B, Table 1). SIC innervation by fibres from other control areas is diverse (Table 1): a large number of labelled fibres innervating the rostral part of the SIC is found after a PHA-L injection into the LHA/ PFX (Fig. 8A). A moderate number of labelled fibres in the SIC is found after an injection into the DHA/

next page:

Figure 4. Schematic sagittal "flow charts" of efferent fibre streams after PHA-L injections in the intermediate hypothalamic area (3B), dorsal part of the ventromedial nucleus and dorsomedial nucleus (3C) and lateral hypothalamic area (3D). For abbreviations used in 4A: see list of abbreviations.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MeA
ot
Arc
MPB
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ME
LaA
BaA
BSTIA
VPM
DPM
NTS
A5
DMV
AHiA
RMg
VLM

PVH and a sparse number after an injection into the AHA (R152) (Table 1). The IHA projects moderately to the **substantia innominata, pars sublenticularis** (SIL) (Figs. 6B,C). The number of fibres originating in control areas projecting to the SIL is less than from the IHA (Table 1). The VMH injections revealing a large number of labelled fibres in the SIL included many labelled neurons dorsal to the VMH (Table 1)(R17, R53). Other VMH injections resulted in only small numbers of labelled fibres in this area.

Fibres enter the **amygdala** through the SIL, through the supraoptic tract and through the stria terminalis (st) (Fig.4,5). Within the amygdala, most fibres from the IHA are confined to its medial parts (Figs. 6C,D).

The **medial amygdaloid nucleus** (MeA) is moderately innervated by IHA fibres, but sparsely by fibres from the ventrolateral VMH. The largest number of labelled fibres in the MeA was found after an injection in the PVH/ DHA (Table 1). The injection in the lateral hypothalamic area/ perifornical nucleus did not result in labelled fibres in the MeA.

A considerable number of smooth fibres traversing the lateral parts of the amygdala and the piriform cortex (Pir) is found after injections into the VMH (Figs. 7C,D,E). Other hypothalamic areas that have been studied, do not appear to project to the lateral parts of the amygdala. After injections into the VMH labelled fibres are found travelling dorsally along the central amygdaloid nucleus (CeA) to lateral, thus innervating the **lateral amygdaloid nucleus** (LaA) (Figs 6,7E). In our study, the VMH was found to be the single hypothalamic area projecting to the lateral amygdaloid nucleus (Table 1).

The **central amygdaloid nucleus** (CeA) receives a sparse number of fibres from the IHA and a moderate number from the VMH (Figs. 6,7, Table 1). The PVH/ DHA projects moderately to the CeA. No projection from the LHA/ PFX to this nucleus is found (Table 1, Figs. 8D,E).

A large number of labelled varicose fibres is present in the intra- amygdaloid part of the bed nucleus of the stria terminalis (BSTIA) in virtually all series that have been examined (Table 1). Some labelled fibres are also present in the amygdalo-hippocampal area (AHiA) (Fig. 6,7-F, Table 1). Labelled fibres are sarsely found within the stria terminalis after an injection in the LHA/ PFX, but no labelled fibres are found in the BSTIA and AHiA (Fig. 8F).

The lateral ascending fibre stream

Within the lateral preoptic area efferent hypothalamic fibres are running rostrally towards the septum forming the lateral ascending fibre stream (stream 2, fig.5). Along their way within the lateral preoptic area, sparse varicosities are found until the fibres enter the septum.

Efferent fibres from different hypothalamic areas appear to terminate in different parts of the **lateral septum** (Figs. 6,7,8A, Table 2). Different parts of the **intermediate part of the lateral septal nucleus** (LSI) appear to receive afferent projections from different hypothalamic areas as well (Fig.9, Table 2). Labelled efferent fibres from the hypothalamic "attack area" (HAA) are mainly found in the lateral and ventral parts of the LSI (Table 2). Injections in the intermediate hypothalamic area (IHA) specifically result in a large number of labelled fibres innervating the dorsolateral part of the LSI. Terminals in this area originating in the IHA completely surround the septal neurons, forming pericellular baskets (Fig.10). These septal pericellular baskets have only been observed after PHA-L injections into the IHA and not after injections into other hypothalamic areas.

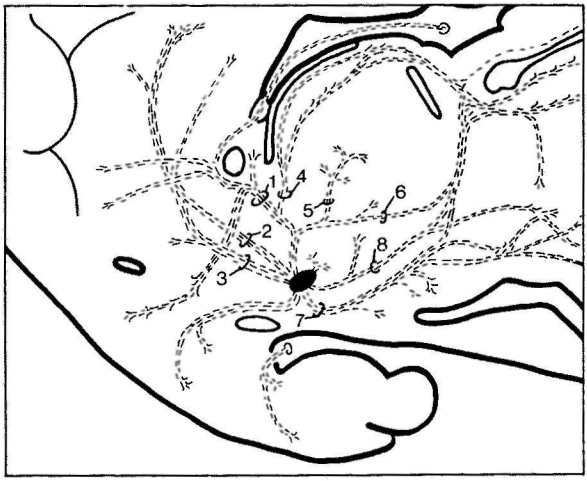
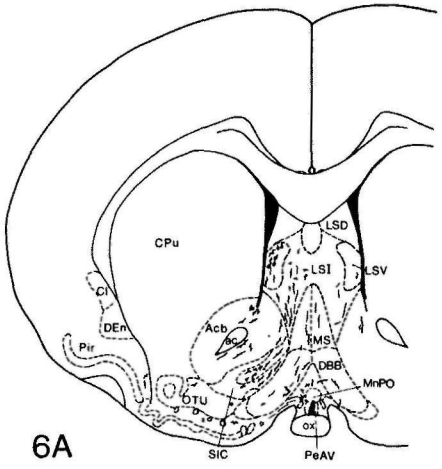


Figure 5. *Detail of figure 4B. Schematic indication of fibre streams after PHA-L injection into the intermediate hypothalamic area.*

Other hypothalamic areas project differently to the septum (Table 2). Fibres from the LHA/ PFX are mainly found in the dorsal part of the lateral septal nucleus (LSD). Fibres from the VMH/ DMH (rat nr. R17) are mainly confined to the medial and ventral parts of the LSI (Fig.9, Table 2). The PVH/ DHA projects largely to the ventral part of the lateral septal nucleus (LSV) and to the ventral part of the LSI and, moderately, to the lateral part of the LSI (Table 2). Fibres originating in the anterior hypothalamic area (R152) are mainly destined for the lateral, ventral and dorsolateral part of the LSI.

The vertical limb of diagonal band of Broca (DBB) receives afferent projections from all hypothalamic areas that have been examined, except from the ventrolateral VMH (R32, R39, Table 1). Labelled fibres in the DBB are running towards the medial septal nucleus (MS). The MS is moderately innervated by IHA fibres (Fig. 6A). The VMH, LHA/ PFX and AHA project only sparsely to the MS (Table 1).

Figures 6, 7, 8. *Drawings of efferent projections of an IHA injection (Fig.5, rat R345), a VMH/ DMH injection (Fig.6, rat R17) and a LHA injection (Fig. 7, rat R336). The δδ in Figs. 6A and 6B indicate septal pericellular baskets (see text). For abbreviations: see list of abbreviations.*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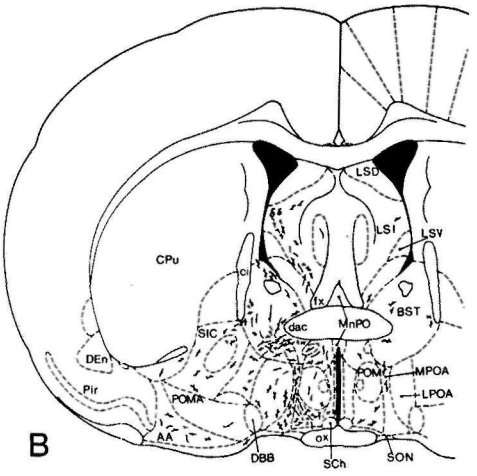
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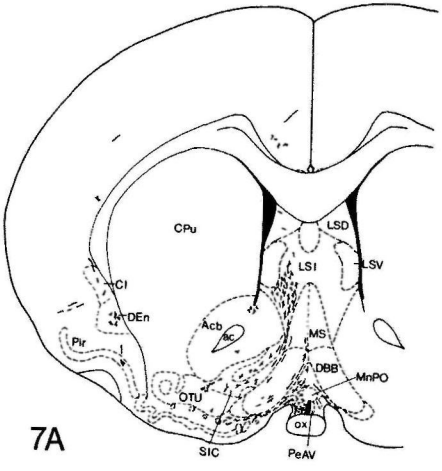
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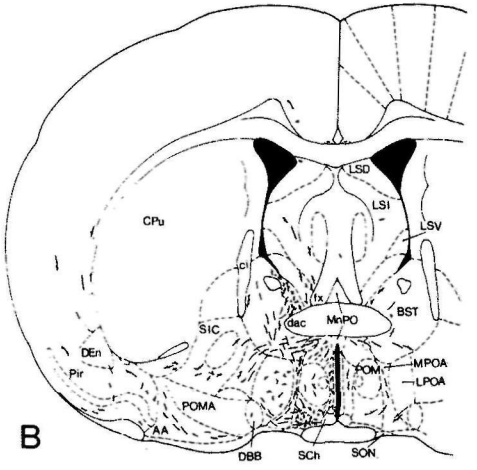
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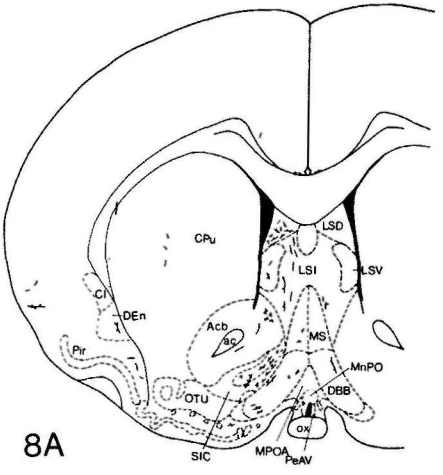
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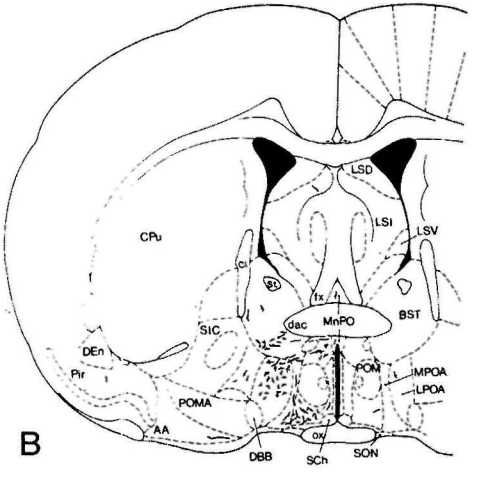
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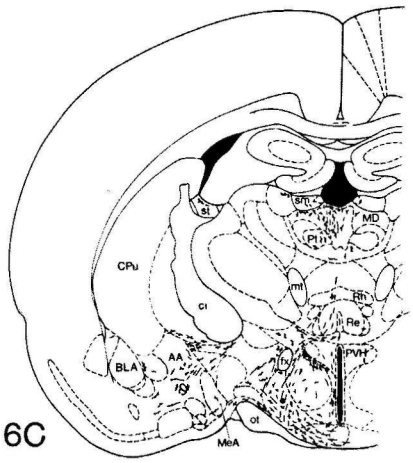
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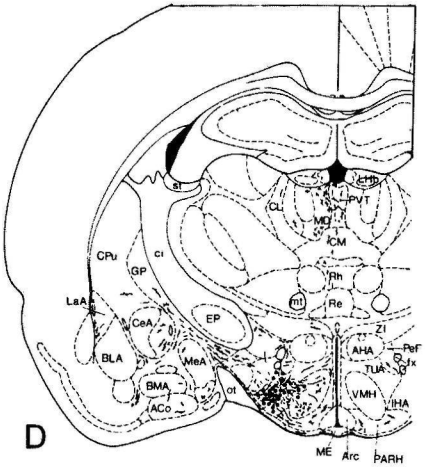
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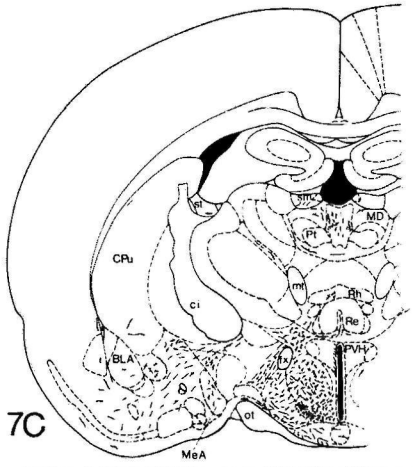
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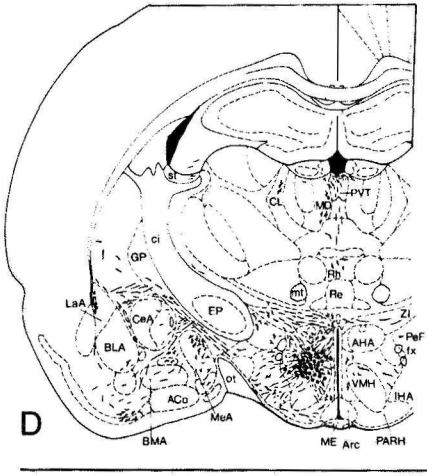
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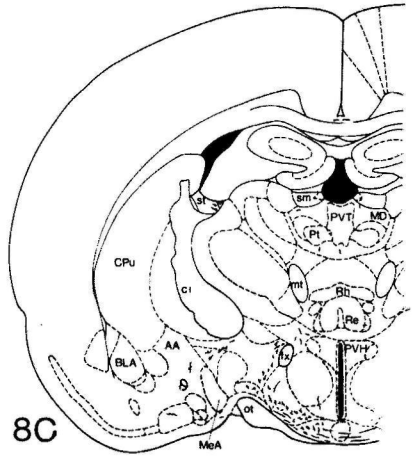
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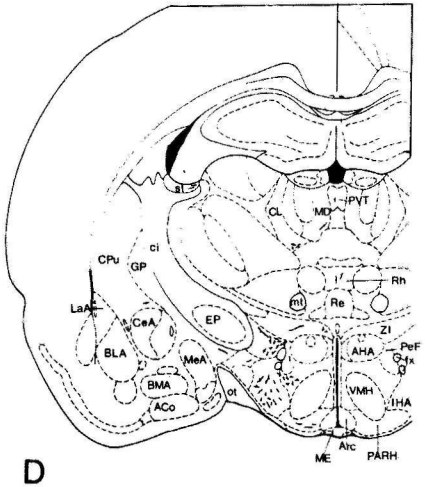
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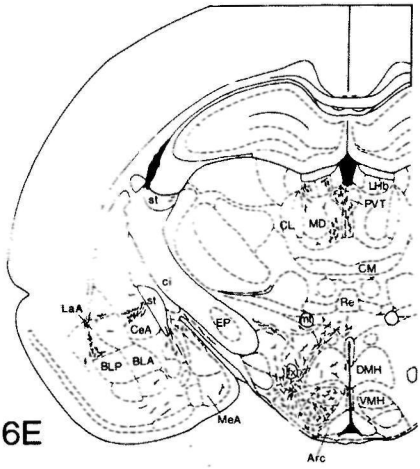
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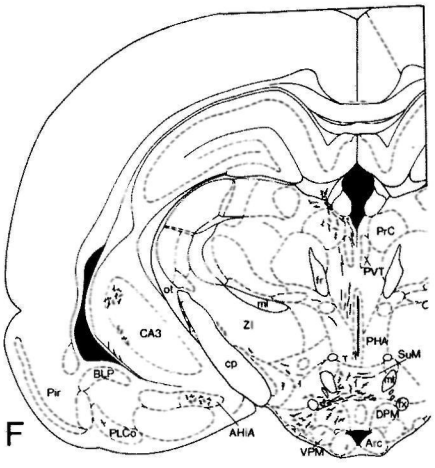
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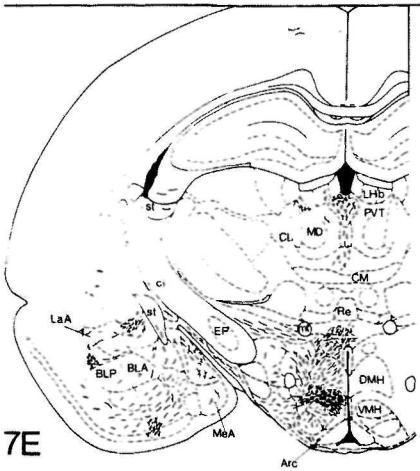
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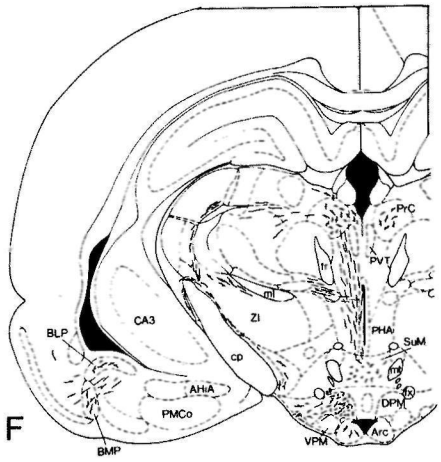
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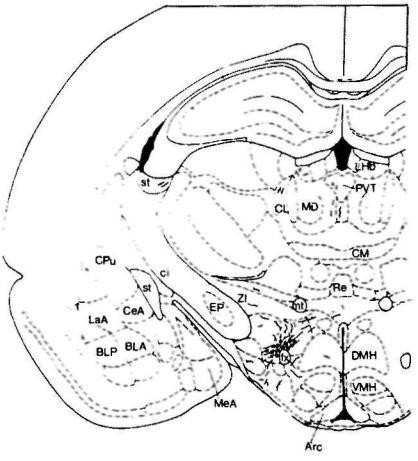
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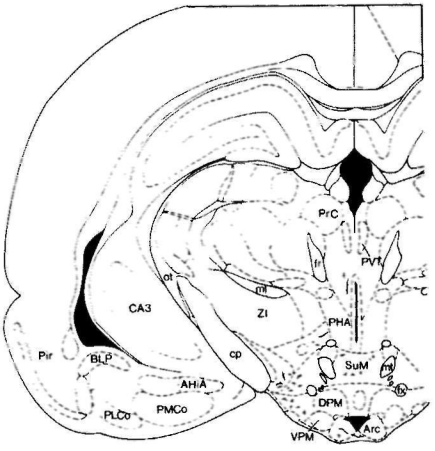
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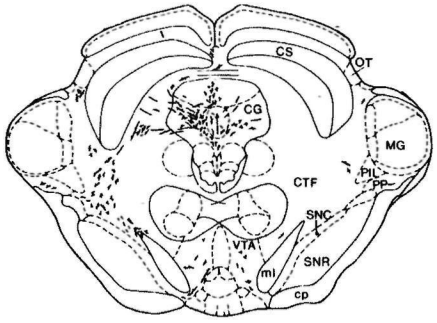
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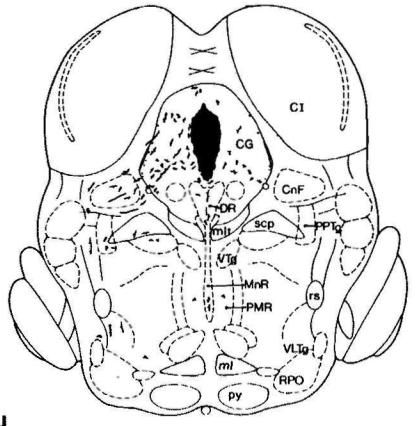
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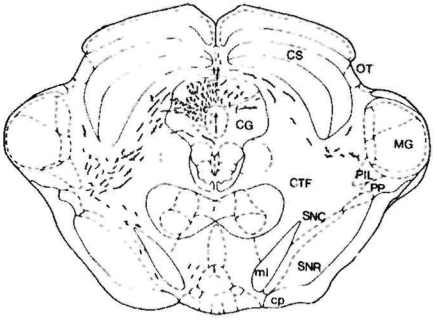
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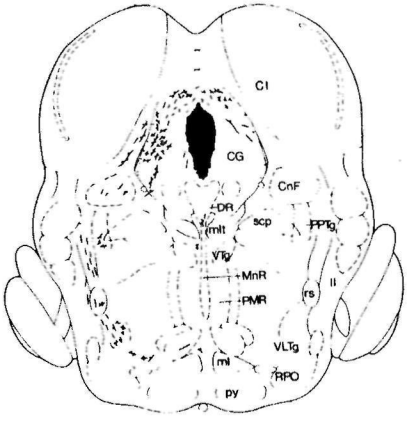
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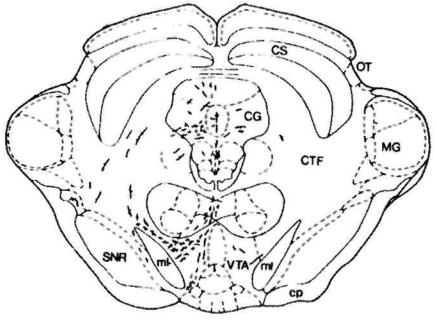
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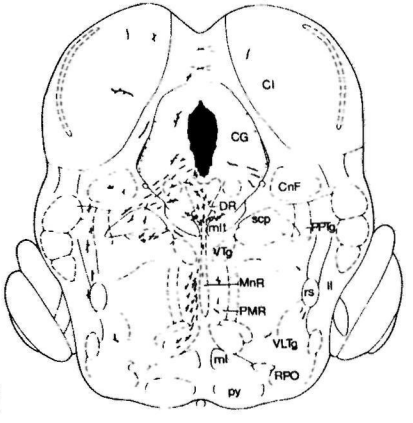
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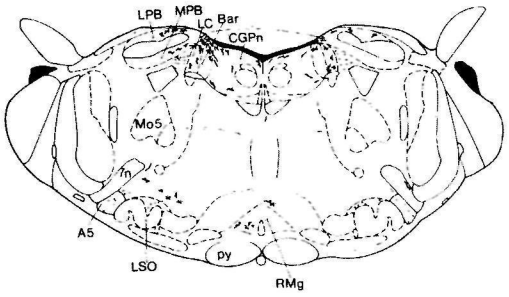
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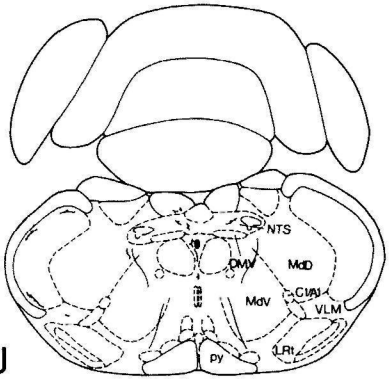
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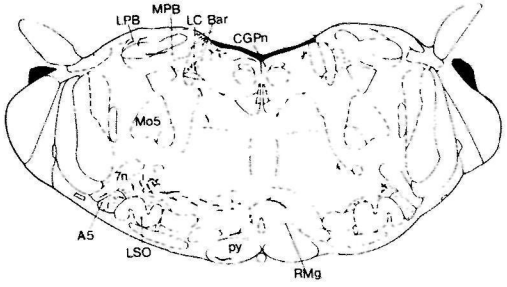
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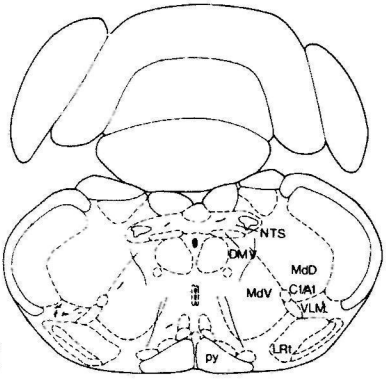
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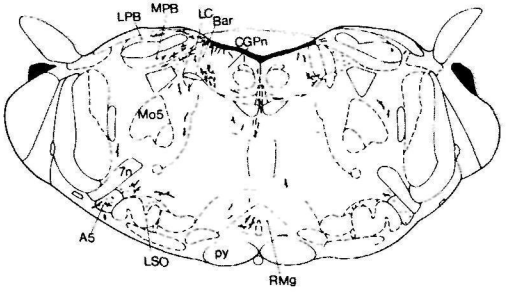
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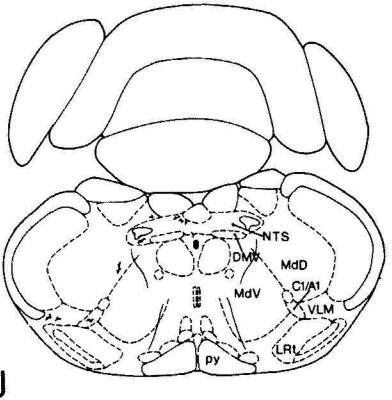
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The medial ascending fibre stream

Fibres in the medial ascending fibre stream (stream 3, Fig.5) pass through the medial preoptic area (MPOA), ventral to the medial preoptic nucleus (POM). Fibres from the IHA pass mainly through the lateral preoptic area (LPOA), following the lateral ascending fibre stream. A smaller number of fibres is found following the medial ascending stream. Fibres from the VMH, on the other hand, are situated in the MPOA, following the medial ascending fibre stream, more than in the LPOA, following the lateral ascending stream. A large contingent of the fibres from the IHA running within the medial fibre stream appears to turn laterally and to join fibres from the lateral fibre stream towards the septum (Fig.4B). Other fibres remain in the ventral position and apppear to terminate within the medial preoptic area.

Thalamic fibre streams.

Efferent hypothalamic fibres enter the thalamus via two different routes: the largest number of fibres enters the thalamus rostrally at the rostral pole of the thalamic paraventricular nucleus, medially to the stria medullaris (sm) and runs caudally within the dorsomedial thalamus, following the dorsal thalamic fibre stream (stream 4, Fig.5). Other fibres enter the thalamus in the medial plane, following the ventral thalamic fibre stream (stream 5, Fig.6).

The dorsal thalamic fibre stream.

Fibres from the dorsal thalamic fibre stream cluster within the middorsal part of the thalamus and run caudally to the transition area of diencephalon and mesencephalon. All along their caudal course, many varicosities are present. Some labelled fibres are found within the stria medullaris. From this fibre stream, dorsomedial thalamic structures are innervated. The **parataenial thalamic nucleus** (Pt) receives many efferent fibres from the IHA, while fibres from other hypothalamic areas innervate this nucleus only sparsely (Fig. 6C, Table 1). The **thalamic paraventricular nucleus** (PVT) contains labelled fibres in all series investigated (Figs. 6-, 7-, 8C-E). In general, more labelled fibres in the PVT were found if more hypothalamic neurons were labelled. The **mediodorsal thalamic nucleus** (MD) is strongly innervated by IHA fibres (Figs. 6C-E, Table 1). These fibres are mostly situated in the medial part of the MD. Other hypothalamic areas appear to project only sparsely to the MD (Figs 7-, 8C-E, Table 1). The medial part of the **central thalamic nucleus** (CM) receives projections from all hypothalamic areas that have been examined (Figs. 6-8E, Table 1). The lateral part of the central thalamic nucleus (CL) appears in general to be not or only sparsely innervated by fibres from hypothalamic areas, except by the PVH/DHA and LHA/ PFX: these areas project moderately to the CL (Fig. 8, Table 1). Labelled fibres without varicosities within the stria medullaris (sm) were found after most injections into the intermediate hypothalamic area (IHA), after some injections into the ventromedial hypothalamic nucleus (VMH) but not after injections into the PVH/ DHA, AHA and LHA/ PFX (Table 1). At caudal levels, a part of the fibres from the dorsal thalamic fibre stream cluster in the medial part of the lateral habenular nucleus (LHb) (Figs 6,7E). Other fibres remain positioned ventral to the LHb and inside the paraventricular thalamic nucleus. Virtually all hypothalamic areas appear to innervate the LHb (Table 1). At the transition between diencephalon and mesencephalon, fibres from the dorsal thalamic fibre stream cross and/ or intermingle with fibres descending through the posterior hypothalamic area (see below) (Fig.4).

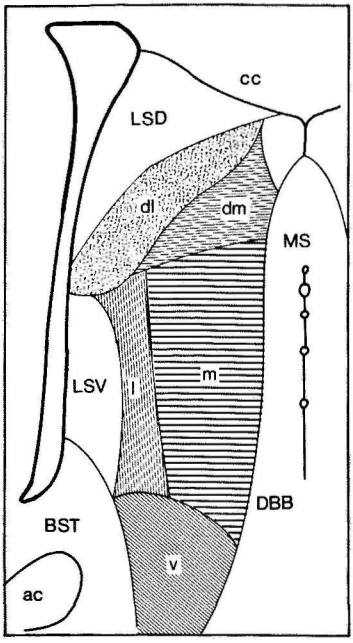

Table 2

		LSD	LSV	dl	dm	l	m	v
IHA	15	x	xx	xxx	x	xxx	xx	xxx
	345	o	x	xxx	x	xx	x	xx
	334	x	xx	xxx	x	xxx	xx	xx
	207	x	xx	xxx	o	xxx	x	xx
VMHvl	39	o	x	x	x	x	xx	xx
	32	o	x	x	x	x	xx	xx
VMH	53	x	xx	o	x	xxx	xxx	xxx
	282	x	x	o	x	xx	x	xx
VMH/DMH	17	x	x	x	o	xx	xx	xxx
PVH/DHA	111	x	xxx	x	x	xx	xx	xxx
AHA	152	x	xx	xxx	x	xx	xxx	xx
LHA/PFX	336	xxx	x	x	x	xx	x	xx

Figure 9. *Diagrammatic transverse section through the septal region of the rat. The intermediate part of the lateral septal nucleus has been subdivided into a dorsomedial (dm), a dorsolateral (dl), a lateral (l), a medial (m) and a ventral (v) division.*

Table 2. *Semiquantitative indication of the innervation of different parts of the lateral septal nucleus. o: no innervation; x: sparse innervation; xx: moderate innervation; xxx: large innervation. For abbreviations: see figure 9.*

The ventral thalamic fibre stream.

Smooth fibres from the hypothalamus turn dorsally and enter the thalamus through the midline via the ventral thalamic fibre stream (stream 5, Fig.5). Fibres within this stream innervate the **nucleus reuniens** (Re) and the **nucleus rhomboideus** (Rh), branching with varicosities in these nuclei. Both nuclei receive a sparse or moderate projection from all hypothalamic areas that have been studied (Figs. 6,7,8C-E, Table 1).

Descending fibre streams.

Four major descending fibre streams can be distinghuished: a ventral, a lateral, a dorsomedial and a "zona incerta" fibre stream.

The dorsomedial descending fibre stream.

A contingent of efferent hypothalamic fibres form a cluster dorsal to the third ventricle, forming the dorsomedial descending fibre stream (stream 6, Fig.5). This stream runs through the posterior hypothalamic area towards the mesencephalic central gray (CG), thus joining the fasciculus longitudinalis dorsalis of Schütz. Rostral to the posterior hypothalamic area (PHA), the fibres are mainly varicose, intermingled with smooth fibres. Within the PHA, fibres are mainly smooth with few varicosities. No marked differences between the numbers of fibres from the hypothalamic areas that have been studied, are found. At the transition zone between diencephalon and mesencephalon, fibres from this stream join the fibres from the dorsal thalamic fibre stream (stream 4, Fig.5) and enter the central gray. In most series that have been examined, many varicosities and terminal arborizations are found in the **precommissural nucleus** (PrC) (Figs. 6-7F).

The ventral descending fibre stream.

The ventral descending fibre stream (stream 7, Fig.5) is situated in the ventral parts of the hypothalamus, within the IHA and ventral to the VMH. From this fibre stream fibres innervate the ventromedial hypothalamic nucleus (VMH), the hypothalamic arcuate nucleus (Arc) and the median eminence (ME).

The **ventromedial hypothalamic nucleus** (VMH) receives a large projection from the intermediate hypothalamic area (IHA). The hypothalamic paraventricular nucleus, the lateral and the anterior hypothalamic areas project to the VMH considerably less than the IHA (Table 1). The **arcuate nucleus** is sparsely innervated by efferent fibres from the IHA, LHA and AHA, but largely by efferent fibres from the PVH/DHA. The VMH projects moderately to the arcuate nucleus (Figs. 6,7,8D-F, Table 1). Labelled fibres in the **median eminence** (ME) were only found after PHA-L injections into the PVH/ DHA (R111) and into the anterior hypothalamic area (R152) (Table 1).

Some fibres from the ventral descending fibre stream turn dorsally and innervate the **dorsomedial hypothalamic nucleus** (DMH). The DMH is only strongly innervated by fibres from the PVH/ DHA (Table 1). PHA-L injections into the intermediate hypothalamic area (IHA), lateral hypothalamic area (LHA)/ perifornical nucleus (PFX) and ventromedial hypothalamic area (VMH) resulted in only a sparse number of labelled fibres in the DMH (Figs. 6,7,8E, Table 1). The anterior hypothalamic area projects moderately to the DMH (Table 1).

Further caudally, the ventral descending fibre stream enters the mammillary body. Fibres from this stream innervate the **ventral and dorsal premammillary nuclei** (Figs.4,5). The dorsal premammillary nucleus (DPM) contains more labelled fibres than the ventral premammillary nucleus (VPM) in all series that have been studied (Figs. 6,7,8F, Table 1). The IHA projects moderately or largely to the DPM (Table 1). Except

Figure 10. *Photomicrograph of the septal innervation after a PHA-L injection into the intermediate hypothalamic area. Scale bar: 100 μm. A: Detail of pericellular baskets in the dorsolateral corner of the intermediate part of the lateral septal nucleus.*

for the ventrolateral VMH and LHA/ PFX, which project sparsely to the DPM, all hypothalamic injections resulted in moderate or large projections to the DPM (Table 1).

Many fibres from the ventral descending fibre stream turn dorsally to join the fibres traversing the posterior hypothalamic area (PHA) towards the central gray (Figs. 6,7,8 F). Other fibres stay in the ventromedial part of the hypothalamus and run caudally through the ventral tegmental area and, further caudally, through the ventromedial parts of the brainstem including the raphe magnus (see below). This part of the ventral descending fibre stream is considered as a part of the ventromedial descending continuation of the medial forebrain bundle [26].

The lateral descending fibre stream.

The lateral descending fibre stream (stream 8, fig. 5) traverses the lateral hypothalamic area (LHA) and forms a part of the medial forebrain bundle [49]. Only few efferent fibres from the intermediate hypothalamic area and the ventromedial hypothalamic nucleus are found in this stream (Figs 6-, 7D-E). Control injections resulted in general in more labelled fibres in this fibre stream and in the LHA than injections in the IHA and VMH (Table 1). A large contingent of the fibres in the lateral descending fibre stream turns laterally and enters the supraoptic tract, running towards the amygdala (see above). Further caudally, fibres from this stream either turn dorsally towards the central gray or stay ventrolaterally to innervate the ventral aspects of the central tegmental field (CTF), running further caudally as a ventrolateral descending continuation of the medial forebrain bundle [26].

The "zona incerta fibre stream".

This stream is not indicated in figures 4 and 5, but consists of fibres running through the zona incerta. Fibres within this stream originate from most hypothalamic areas but the contribution of different hypothalamic areas to this stream is not the same. After IHA injections, only a sparse number of labelled fibres are found in this stream. Efferents from other hypothalamic areas contribute with moderate numbers of fibres, except for the lateral hypothalamic area/ perifornical nucleus, which has only sparse fibres in this stream. Fibres in the "zona incerta" stream enter the zona incerta medially and run caudally towards the central tegmental field (CTF) in the mesencephalon. Most fibres are positioned in the ventral aspects of the ZI, close to the cerebral peduncle (cp) (Figs. 6-, 7-, 8E-F). Within the CTF, these fibres join other descending fibres from the lateral and ventral descending fibre streams (see above).

Brainstem projections.

Caudal to the diencepalon, the fibre streams regroup into three descending brainstem fibre streams, viz. the dorsal, the ventromedial and ventrolateral brainstem fibre stream.

The dorsal brainstem fibre stream.

In the mesencephalon, fibres from the dorsal brainstem fibre stream descend via the central gray, as a part of the dorsal longitudinal bundle of Schütz (Figs. 4, 5). Many

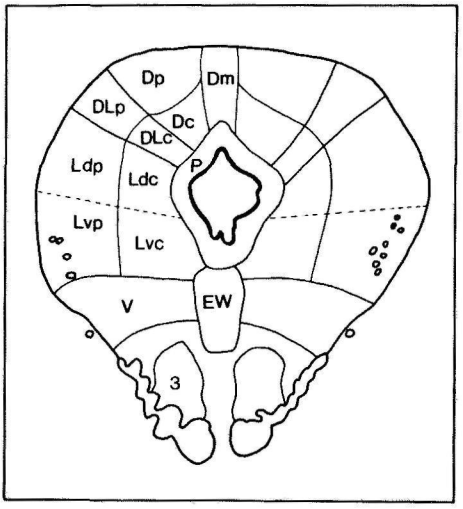

Table 3

		Dm	Dp	Dc	DLp	DLc	Ldp	Ldc	Lvp	Lvc	V	P
IHA	15	o	o	xxx	o	o	xx	xx	o	o	x	xx
	345	o	x	xx	o	o	xxx	xxx	o	o	x	x
	334	o	xx	xxx	o	o	x	x	o	o	x	o
	207	xxx	x	xxx	o	o	x	xxx	o	xxx	x	x
VMHvl	39	o	x	x	o	o	x	xx	o	o	o	x
	32	o	x	x	o	o	x	xxx	o	o	o	x
VMH	53	xxx	xxx	xxx	o	xxx	xx	xxx	o	xxx	xx	xx
	282	xxx	o	xxx	o	xx	xx	xx	o	o	o	x
VMH/DMH	17	xx	x	xx	o	xxx	x	xx	o	o	x	x
PVH/DHA	111	o	o	xxx	o	o	o	x	o	xxx	x	xx
AHA	152	xxx	o	xxx	xxx	xxx	o	x	o	x	x	x
LHA/PFX	336	o	o	o	o	o	o	o	xxx	xxx	x	o

Figure 11. *Line drawing of the mesencephalic central gray, indicating the subdivisions of the CG, according to [73]. D: dorsal part, DL: dorsolateral part; L: lateral part; V: ventral part; P: periventricular part; EW: Edinger Westphall nucleus; 3 motor nucleus third cranial nerve. Further subdivisions have been indicated as m: medial; d: dorsal; v: ventral; p: peripheral and c: central.*

Table 3. *Semiquantitative indication of the innervation of different parts of the mesencephalic central gray. o: sparse innervation; x: sparse innervation; xx: moderate innervation; xxx: large innervation. For abbreviations: see figure 10.*

varicosities are found in this area. A number of fibres leaves this fibre stream laterally and innervates the dorsal aspects of the central tegmental field. At the level of the inferior colliculus (CI), fibres leave the CG laterally to innervate the cuneiform nucleus (CnF) and the pedunculopontine tegmental nucleus (PPTg) (Figs. 6-, 7-, 8H). Other fibres from this stream innervate a number of brain areas close to the CG, like locus coeruleus, nucleus of Barrington and parabrachial nuclei (Fig.4). A number of fibres in this area is found below the lateral corner and perpendicular to the wall of the fourth ventricle (Figs. 6-, 7-, 8I). Sometimes these fibres are positioned between the cells of the ependymal layer. This fibre stream runs caudally towards the nucleus of the solitary tract (NTS).

Within the **central gray** (CG), a differential innervation of parts of the CG by different hypothalamic areas can be distinghuished. The cytoarchitectonic subdivision of the CG have recently been reinvestigated [73]. This subdivision has been used to investigate the spatial organization of CG innervation by the hypothalamic injection sites under study. On the basis of the distribution of PHA-L labelled fibres, the lateral part of the CG can be

further subdivided into a dorsal and a ventral part (Fig. 11). A semiquantitative listing of the hypothalamic innervation of the different parts of the CG is indicated in Table 3. Efferent projections from the hypothalamic attack area (HAA), *i.e.* the intermediate hypothalamic area and the ventrolateral pole of the ventromedial hypothalamic nucleus, are mainly found in the dorsal part of the CG (CGDp, CGDc) and the dorsal part of the lateral CG (CGLdp, CGLdc). Efferent fibres from the lateral hypothalamic area/ perifornical nucleus are concentrated in the ventral parts of the lateral CG, while efferent projections from the anterior hypothamamic area are concentrated in the dorsolateral part of the CG. The PVH/ DHA projects mainly to the central aspects of the dorsal and lateroventral parts of the CG (Table 3).

The pontine central gray (CGPn) is only sparsely innervated by IHA and VMH fibres, whereas many fibres from the PVH/ DHA are found in this area. The AHA and LHA project moderately to the CGPn (Table 1, Fig.8I).

Within the **cuneiform nucleus** (CnF) moderate numbers of labelled fibres are found in all series (Figs. 6,7,8H, Table 1). The **peduncolopontine tegmental nucleus** (PPTg) receives afferent projections from most hypothalamic areas that have been investigated as well. In general, medially placed injection sites, *i.e.* within PVH/ DHA, AHA and VMH, resulted in more labelled fibres in the PPTg than laterally placed injection sites, *i.e.* within IHA and LHA (Table 1).

With the exception of the hypothalamic paraventricular nucleus and dorsal hypothalamic area (PVH/ DHA), which project largely to the **locus coeruleus** (LC), all other hypothalamic areas studied so far project sparsely or moderately to this nucleus (Table 1). The **nucleus of Barrington** (Bar) is sparsely innervated by IHA fibres and not by VMH fibres, except for case R17, in which a moderate number of labelled fibres is present (Figs. 6,7I, Table 1). PVH/ DHA fibres innervate Bar moderately (Table 1). The LHA/ PFX projects largely to the nucleus of Barrington, especially to the ventral part (Fig.8I).

The innervation of the **lateral and medial parabrachial nuclei** (LPB and MPB) by fibres from different hypothalamic areas was variable (Table 1). PHA-L injections within the intermediate hypothalamic area (IHA) and ventromedial hypothalamic nucleus resulted in sparse numbers of labelled fibres in the LPB and MPB (Figs. 6-, 7I, Table 1). The PVH/ DHA projects largely to the LPB and MPB (Table 1). The LHA projects moderately to both parts of the parabrachial nuclei (Fig. 8I, Table 1).

In general, no innervation is found of the **nucleus of the solitary tract** (NTS) by efferent fibres from the hypothalamic "attack area" (HAA) (Table 1). Parts of the VMH outside the HAA project sparsely to the NTS. The PVH/ DHA has a large projection to the NTS. The anterior and lateral hypothalamic areas appear to project sparsely to the NTS (Table 1).

The ventral brainstem fibre stream.

Fibres of the ventral brainstem fibre stream can be considered as part of the ventromedial descending continuation of the medial forebrain bundle, running through the ventral tegmental area [26,51]. Some fibres turn laterally and join the fibres of the lateral descending continuation of the medial forebrain bundle, within the central tegmental field.

Others stay ventromedially and run further caudally towards the raphe magnus. A part of these fibres turns dorsally within the median plane through the median (MnR) and paramedian (PMR) raphe nuclei towards the dorsal raphe nucleus (Figs. 4, 6-8H). Especially the PHA-L injection into the lateral hypothalamic area/ perifornical nucleus resulted in a large number of labelled fibres in the MnR and PMR (Figs. 4, 8G-H).

The **ventral tegmental area** (VTA) is only moderately innervated by fibres from the hypothalamic "attack area" (HAA) (Table 1). These fibres are mainly smooth. Other hypothalamic areas have larger projections to this area. Larger numbers of varicose fibres in this area originate in the PVH/ DHA and in the LHA/ PFX, but their respective localization in the VTA is different: fibres from the PVH/ DHA are situated in the medial and ventral parts of the VTA, while LHA fibres are concentrated in the lateral part of the VTA, dorsomedial to the medial lemniscus (Fig.8G).

Within the **central tegmental field** (CTF), more fibres from the ventral IHA than from the dorsal IHA are found (Table 1). The numbers of labelled fibres in the CTF after injections into the VMH are moderate or large, depending of the number of neurons labelled by the injection (Table 1). PVH/ DHA fibres and LHA fibres innervate the CTF heavily, whereas AHA fibres are only sparsely found in this area (Table 1, Fig. 8G). This difference in innervation could not be attributed to differences in the number of labelled neurons at the injection site.

In general, only minor projections from the hypothalamus to the median and dorsal raphe nuclei are found. The IHA projects moderately to the **median raphe nucleus** (MnR) (Fig.6H). The VMH projects only sparsely to the MnR (Fig 7H, Table 1). However, a large number of labelled fibres in the MnR and paramedian raphe nucleus (PMR) is found after an injection into the LHA/ PFX (Fig. 8H). The **dorsal raphe nucleus** is more extensively innervated by fibres from the ventral IHA (R345, R15) than by fibres from the dorsal IHA (R207/ 334). VMH injections resulted in only sparse numbers of labelled fibres in the DR (Fig.7H, Table 1). A moderate number of labelled fibres is found after injections into the PVH/ DHA and LHA/ PFX (Table 1). The DR is sparsely innervated by AHA fibres.

The **nucleus raphe magnus** (RMg) receives many fibres from the PVH/ DHA (Table 1). All other hypothalamic areas that have been investigated, appear to project sparsely to this nucleus (Figs. 6-, 7-, 8I, Table 1).

The lateral brainstem fibre stream.

The lateral brainstem fibre stream runs caudally and innervates the **ventrolateral tegmentum** (VLTg). In general, only sparse numbers of labelled fibres are found in the VLTg in all series examined (Table 1). Some of the labelled fibres in this area turn dorsally towards the pedunculopontine tegmental nucleus (PPTg). Other fibres stay in the ventrolateral position and run through the noradrenergic A5 area towards the ventrolateral medulla (VLM) (Fig. 4).

The **noradrenergic A5** region is only sparsely innervated by efferent fibres from the dorsal IHA and not by fibres from ventral IHA (Table 1). Other hypothalamic areas project sparsely or moderately to the A5 region, except for the PVH/ DHA, which sends a

large efferent projection to this area (Table 1). Labelled fibres in the **ventrolateral medulla** (VLM) are found after injections into the IHA in one case only (Fig. 6J, Table 1). Fibres from the VMH are only sparsely found in the VLM (Table 1). The LHA/ PFX does not project to this area. Instead, there is a large projection from the PVH/ DHA to this area (Table 1).

Discussion.

Technical remarks.

The advantage of using *Phaseolus vulgaris* leucoagglutinin (PHA-L) in the study on the efferent projections of specific behaviour-related brain areas is twofold: the labelled neurons of origin are clearly visible and the characteristics of the fibres "en route" can be observed [24]. Since the neurons within the hypothalamic "attack area" responsable for the behavioural effect have not been identified in some way and the hypothalamic "attack area" can not be regarded as a cytoarchitectonically defined area, a precise localization of the labelled neurons of origin is necessary.

Delineation of the hypothalamic "attack area".

The hypothalamic "attack area" (HAA) does not coincide with a distinct cytoarchitectonic entity. Using a large number of electrode placements where attack behaviour was elicited with electrical stimulation [32,34,38], the HAA was delineated as an area including most of the intermediate hypothalamic area (IHA) and the ventrolateral pole of the ventromedial hypothalamic nucleus (VMH). However, since electrical stimulation may activate a combination of passing fibres and neuronal cell bodies, it is possible that the area that has been delineated in this way involves parts where passing fibres have been stimulated and parts where cell bodies were stimulated. In a behavioural study using chemostimulation of ventral parts of the hypothalamus, attack behaviour has been elicited [57]. This indicates, that at least in some parts of the hypothalamus neuronal cell bodies are involved. The positive injection sites in this study were all placed lateral to the ventromedial hypothalamic nucleus within the HAA, as it is delineated in the present study. The small number of injection sites, however, did not allow to draw conclusions about the extent of the hypothalamic area where attack can be elicited by stimulation of neuronal cell bodies [57].

By comparing the efferents of the intermediate hypothalamic area with those of the ventrolateral pole of the ventromedial hypothalamic nucleus, many differences have been found. This may indicate that the neurons labelled after these injections, are not necessarily all involved in the regulation of attack behaviour. Since hypothalamic neurons that are effectively involved in attack behaviour have not been identified in some other, more specific way, the differences between the efferents of the IHA and ventrolateral VMH suggest, that the HAA in fact consists of a smaller area than delineated in figure 2. Neurons in the ventrolateral pole of the VMH have some characteristics that are different from the neurons in the IHA: within the ventrolateral pole of the VMH, neurons containing substance P and enkephalin have been found [27,39]. This part of the VMH has also neurons containing oxytocin receptors [67] and estrogen receptors [18]. Such neurons are not, or much less frequently, found in the IHA. A further examination of the neurons that form

the HAA using different techniques is therefore necessary. A large number of positive electrode placements is situated in the IHA. Moreover, the number of effective electrode placements within the VMH is much smaller. Therefore we tend to consider the efferent projections from the IHA as more related to attack behaviour than the efferents from the ventrolateral part of the VMH. Consequently, in the discussion on the specificity of efferent projections of the hypothalamic "attack area" the emphasis will be put on the efferents of the intermediate hypothalamic area.

Efferent fibre "streams" and their characteristics.

Efferent fibres from hypothalamic areas do not form well- determined bundles or fascicles [56]. Within the hypothalamus, efferent fibres spread out in various directions, clustering in some parts of the hypothalamus, but often intermingling with other sets of fibres. In the description of the paths followed by these fibres towards other parts of the brain, a number of fibre "streams" were identified. Three ascending and four descending fibre "streams" could be distinghuished. The ascending streams were designated as a dorsal, a lateral and a medial stream. The dorsal ascending fibre stream innervates the bed nucleus of the stria terminalis, the lateral fibre stream runs through the lateral preoptic area towards the septal complex and the ventral ascending fibre stream runs through the medial preoptic area. Descending fibres were found to form four streams, a dorsomedial, a ventral, a lateral and a "zona incerta" fibre stream. Caudally, these fibre streams regroup into three "brainstem fibre streams": a mediodorsal stream, contributing to the fasciculus longitudinalis dorsalis of Schütz, a ventromedial stream, as a part of the caudal ventromedial continuation of the medial forebrain bundle, and a ventrolateral stream, as a part of the caudal ventrolateral continuation of the medial forebrain bundle [26,51].

Each fibre stream does not contain the same number of fibres from each hypothalamic area. Ascending fibres from the HAA are situated more extensively in the lateral and dorsal ascending fibre stream than in the ventral descending fibre stream. Descending fibres from the HAA are found in the dorsomedial and lateral descending fibre streams and only sparsely in the ventral descending fibre stream and "zona incerta" fibre stream. This indicates, that the projections to the lateral septum, bed nucleus of the stria terminalis and the central gray are the most important efferent connections of the HAA.

Within each of these streams, many "en passant" varicosities are found, although the number of varicosities differ when different areas of passage are compared. Since these varicosities may contain synaptic specialisations, many brain areas may actually be innervated while being traversed by these fibre streams [75].

Efferent projection areas: preferential innervation by the HAA.

The dense projection from the IHA to the septum has been reported before [71]. Within the septum, a spatial organization of hypothalamic efferents can be observed, showing that fibres from different hypothalamic areas innervate different parts of the septum. The existence of a spatial organization of hypothalamic efferents to the septum has been suggested before in the rat [40] and guinea pig [64]. In addition, a spatial organization of septal efferents within the hypothalamus has been reported as well [43,65,70]. The present study clearly shows that IHA fibres project preferentially to the dorsolateral aspect of the intermediate part of the lateral septal nucleus. This innervation is characterized by

specialized "pericellular baskets", that appear to surround single septal neurons. The baskets were found only after PHA-L injections into the intermediate hypothalamic area and not after injections in other hypothalamic areas. Therefore we consider these baskets as specifically related to the "attack area". Septal pericellular baskets, located in the same part of the lateral septal nucleus, have been reported to contain enkephalin, substance P, dopamine or serotonin [21,29], but their possible hypothalamic origin has not been investigated so far.

The substantia innominata is in the present study subdivided into a subcommissural (SIC) and a sublenticular (SIL) part. Hypothalamic innervation of these areas has been reported previously [25]. In our study, the subcommissural part of the substantia innominata is innervated by all hypothalamic areas that have been examined. Except for the lateral hypothalamic area/ perifornical nucleus, which projects more to the SIC than to the SIL, the hypothalamic areas investigated were found to project to both parts of the substantia innominata, without clear quantitative differences.

The anterior amygdaloid area appears to be more densely innervated by hypothalamic areas medial to the fornix, i.e. the VMH, DMH and PVH, than by the intermediate and lateral hypothalamic areas. Interestingly, the lateral amygdaloid nucleus was only innervated by fibres from injection sites that included parts of the VMH. This connection appears to be reciprocal for the anterodorsal part of the VMH, but not for the ventrolateral part of the VMH [41]. The hypothalamic areas, that have been investigated in the present study, project only moderately to the medial amygdaloid nucleus (MeA). The anterior part of the MeA was suggested to be more densely innervated by dorsal VMH neurons than by ventral VMH neurons [41]. Such a differential innervation was not found in the present study.

Hypothalamic projections to the thalamus have been reported before [14]. In the present study, innervation of the mediodorsal thalamic nucleus (MD) was originating from a large number of neurons within the IHA. Other hypothalamic areas appear to have weaker projections to the MD. In cats, the largest number of hypothalamic neurons projecting to the mediodorsal thalamic nucleus was reported to be situated in the medial part of the lateral hypothalamic nucleus, corresponding with the IHA [53]. The present report indicates that this differential innervation of the MD is apparent in rats as well, with the largest hypothalamic projection arising from the intermediate hypothalamic area. The parataenial thalamic nucleus is also innervated by hypothalamic areas, again with the largest projection arising from the intermediate hypothalamic area. Taken together, the mediodorsal and parataenial nuclei of the thalamus receive a strong hypothalamic input, especially from the hypothalamic "attack area".

Within the mesencephalic central gray (CG) a spatial organization of hypothalamic projections has been distinghuished, as reported before [72]. Interestingly, fibres from both components of the hypothalamic attack area, i.e. the IHA and the ventrolateral part of the VMH, cluster in the same parts of the CG, i.e. the dorsal aspect of the lateral part of the CG and the central aspect of the dorsal part of the CG. This innervation of the dorsal aspect of the lateral CG is in agreement with another report on efferent hypothalamic connections involved in agonistic behaviour [42]. This part of the CG receives also projecti-

ons from other hypothalamic areas, in particular from the VMH.

Many other parts of the brain appeared to receive projections from the hypothalamic "attack area" as well, but this innervation either was not different from those of "control injections" (e.g. to the cuneiform nucleus, ventrolateral tegmental area, pontine central gray and nucleus raphe magnus), or it was less extensive than after "control injections" (e.g. to the arcuate nucleus, noradrenergic A5 region, nucleus of the solitary tract and ventrolateral medulla). The possible participation of these areas in the control of hypothalamically induced attack behaviour can therefore not be related to a specific projection from the hypothalamic "attack area". It is possible that these areas have important afferents to the HAA and are in this way involved in the regulation of attack area. One example is the medial amygdaloid nucleus, which appears to project to the hypothalamus and, especially, to the HAA [42].

Putative "attack relevant circuitry".

Various experimental conditions and different terminologies have been employed in the description of agonistic behaviour in the rat. The characteristics of this behaviour have been described as either predatory, affective or flight oriented [4,5,46]. Although these types of agonistic behaviour were previously suggested to have a different localization within the hypothalamus [5,30,54], they are likely to be positioned within the same hypothalamic area, viz. in the intermediate hypothalamic area and the ventrolateral pole of the ventromedial hypothalamic nucleus [32,35]. Differences in behavioural responses may be attributed to variations in stimulus intensity, posture of the opponent and simultaneous stimulation of different behavioural systems [35].

The hypothalamus is part of the neuronal circuit that is involved in agonistic behaviour. Lesioning and stimulation studies have indicated, that other parts of the brain are also involved. The connection of the hypothalamic "attack area" with these brain areas is important in our understanding of the regulation of agonistic behaviour.

The hypothalamic "attack area" projects to a specific part of the lateral septum. Efferents from the septum descend through the medial forebrain bundle specifically through the HAA [70]. This strong reciprocal connection may be involved in the regulation of aggressive behaviour. It is well known, that lesioning the septum results in a type of behaviour, that has been referred to as "septal rage", characterized amongst others by unprovoked attacks upon experimenters and an overall high reactivity towards environmental stimuli [2]. Within the septum, the anterolateral region seems to be a crucial part to destroy in order to obtain this behavioural change [1,58]. Although the exact behavioural responses as described for hypothalamic stimulation and septal rage differ [3,4], both structures may be part of the same attack controlling system, since septal stimulation has been reported to reduce aggressive behaviour [13]. Within the septum, the site involved in the regulation of attack, viz. the anterolateral region, comprises the dorsolateral aspect of the intermediate part of the lateral septum, where fibres from the HAA preferentially terminate.

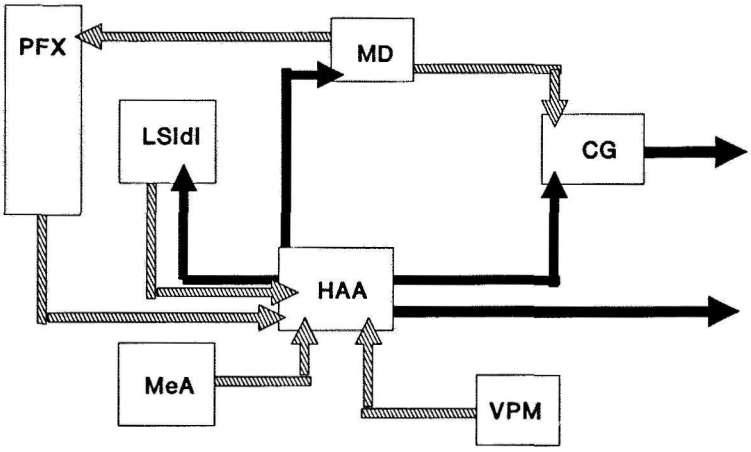

Figure 12. Schematical indication of the major connections of the hypothalamic "attack area" (HAA) in the rat. The solid arrows indicate connections, that have been found in the present study. The hatched lines indicate data from the literature. For abbreviations: see list of abbreviations.

The mediodorsal thalamic nucleus (MD) appears to receive a larger afferent projection from the IHA than from any other hypothalamic area that has been studied so far. The MD plays a facilitatory role in aggressive behaviour [5]. It has been suggested [5] that this role of the MD is exerted through descending fibres that run towards the midbrain central gray, thus following the course of the dorsal thalamic fibre stream, as described in the present study. The specific projection from the hypothalamic "attack area" to the MD may therefore be involved in the regulation of hypothalamically elicited attack behaviour. The strong projection from the MD to the prefrontal cortex may also play an important role. The prefrontal cortex is suggested to inhibit hypothalamically regulated aggression [15]. The neuroanatomical loop from HAA via the MD to prefrontal cortex and back to the HAA may be of interest in future studies on cognitive aspects of agonistic behaviour.

The mesencephalic central gray (CG) in rats has received more attention as a brain site involved in defensive behaviour than in attack behaviour [8,9,16,17]. However, Mos et al. [47] reported the induction of attack behaviour after electrical stimulation of the mesencephalic central gray. Since CG stimulation facilitated hypothalamically elicited attack as well, it was concluded, that the hypothalamic "attack area" and parts of the CG are part of a continuum that forms an "attack relevant circuitry" [47]. However, lesioning the entire CG did not abolish hypothalamically elicited attack and only temporarily increased the threshold current intensity for eliciting attack behaviour in the hypothalamus [48]. It has been suggested, that other pathways are able to compensate for the destructed pathway through the CG [48]. The present study shows that at least three attack relevant descending pathways

occur in the mesencephalon Since the ventromedial pathway, running through the ventral tegmental area, contains only a few fibres from the hypothalamic "attack area", it is our suggestion that the fibre stream running through the ventral part of the central tegmental field is able to compensate for the loss of the behaviourally relevant pathway by the CG destruction Such a compensatory mechanism has also been suggested by Edwards and Adams [19] in the regulation of pain-induced defensive behaviour The positions of the electrodes that elicited attack behaviour [47] agree well with the parts of the central gray that are innervated by fibres from the hypothalamic "attack area" as found in the present study

In cats, other mechanisms have been proposed for the interaction between the hypothalamic "attack area" and central gray, in which the central gray receives "attack associated" afferent input from the anterior hypothalamic area and projects back to the hypothalamic ventromedial nucleus as a positive feedback [59 60]

Electrical and chemical stimulation of the ventral tegmental area (VTA) has been reported to elicit predatory attack in cats and rats [6 7] In our study, only sparse descending fibres arising from the hypothalamic 'attack area" were found in the VTA Ascending fibres from the VTA to the hypothalamus do not extensively innervate the HAA [70]. This suggests, that no direct connections exist between the VTA and the HAA

Lesioning the ventral premammillary nucleus (VPM) appears to increase threatening postures [68] In agreement with other reports [42], only a sparse projection from the HAA to the centre of the VPM was found in the present study However, many passing fibres in the area lateral to the VPM were observed Since the involvement of the VPM in the regulation of agonistic behaviour was studied in lesioning studies, it can not be excluded that destruction of the passing fibres immediately adjacent to the VPM has contributed to the behavioural effect On the other hand, a strong projection from VPM to the hypothalamic attack area exists [42 70] It is therefore likely that the VPM itself is more involved in the afferent control of HAA neuron activity than in the control of "aggression related" projection areas of the HAA The display of threatening postures by VPM- lesioned animals is regarded to be different from attack behaviour elicited by electrical stimulation of the HAA, since the latter is characterized by the absence of threatening postures [31 34 35] If the display of threatening postures in agonistic situations is regulated by the connection from the VPM to the HAA, electrical stimulation of the HAA might overrule the influence of this connection, thus eliciting a direct and uncontrolled attack

Other brain areas that receive efferent projections from the hypothalamic "attack area", appear to be involved in aggressive behaviour as well, like the preoptic area [11], bed nucleus of the stria terminalis [11 44], corticomedial amygdala [12 30 44] and locus coeruleus [20] However, projections to these areas originating from the "attack area" are not different from other hypothalamic projections, either in density or distribution It is possible, that a number of these areas have a preferential efferent projection to the HAA Such a preferential innervation of the HAA has been reported for the medial amygdaloid nucleus [42] and the lateral preoptic area [70], but appears to be absent in the bed nucleus of the stria terminalis [70].

The precommissural nucleus receives afferent projections from all hypothalamic areas, that have been studied Within this nucleus, fibres from descending hypothalamic

fibre streams and the dorsal thalamic fibre stream appear to merge into a single fibre stream entering the central gray. Interestingly, the PHA-L labelled fibres in the precommissural nucleus are highly varicose. Since we are not aware of any report on the possible function of this nucleus, the reason for this "double innervation" is not clear at all.

Serotonergic modulation of hypothalamically elicited attack.

Hypothalamically elicited attack behaviour has been reported to be inhibited specifically by serotonin agonists [33,35,52,69]. Therefore, it is interesting to discuss the brain areas where serotonin can possibly influence "attack related" hypothalamic output. Serotoninergic fibres are found in many parts of the brain, but not in the same density [65a]. Most of the "attack relevant" brain areas, like bed nucleus of the stria terminalis, medial amygdaloid nucleus and central gray, receive a low serotonergic innervation [65a]. The highest density of serotonergic innervation of the central gray appears to be present outside the preferential projection area of the HAA. The HAA itself receives a only sparse serotonergic innervation. The highest density of serotonergic fibres within the hypothalamus is observed lateral to the HAA, within the lateral hypothalamic area [65a].

Serotonergic innervation of the lateral septum is particularly dense [65a]. As mentioned before, hypothalamic "attack area" efferents as well as serotonergic fibres form typical pericellular baskets in the dorsolateral aspect of the intermediate part of the lateral septum. The serotonergic basket- like innervation of the dorsolateral aspect of the intermediate part of the lateral septum may arise from the dorsal raphe nuclei [74]. In addition, many binding sites for serotonergic compounds are found in this specific part of the septum [62,63]. Although we cannot exclude the possibility of the involvement of less distinct projection areas, this part of the septum can be considered as a brain site, where serotonergic modulation of hypothalamically elicited attack behaviour occurs.

Concluding remarks.

Comparing the present findings with our previous report on the efferent connections of behaviourally determined hypothalamic "grooming area" [56], it is concluded that different parts of the hypothalamus, where different kinds of behaviour can be induced, also differ in the distribution of their efferent connections. Every behaviourally determined site is characterized by its specific combination of efferent connections, which are necessary in one way or another for the elicitation of that behaviour. However, there are still numerous gaps in our understanding of the way in which the hypothalamus is capable of regulating these types of behaviour. Behaviourally determined hypothalamic areas show considerable overlap, as do their efferent connections [36,37,38,56,57]. Hypothalamic neurons may have very extended dendrites, which are able to collect information from many different contingents of fibres [45]. Not much is known about the role of specific hypothalamic connections with other brain areas in the regulation of specific types of behaviour, due to the complicated nature of these experiments and to the complicated analysis of elicited or modulated behaviour in these situations. Further study is therefore needed on the exact characterization and identification of neurons involved in hypothalamically elicited types of behaviour, combining anatomical, physiological and behavioural techniques.

The hypothalamic attack area is an important part of the circuitry involved in the regulation of aggressive behaviour and projects to a large number of brain areas (Fig. 12). A number of these areas are known to be involved in aggressive behaviour. More experiments are needed to elucidate the exact function of these connections. Especially the strong preferential connection with parts of the septum is interesting for further research. It is possible that the dorsolateral portion of the intermediate part of the lateral septum projects to other brain areas that are also important for the display of aggressive behaviour, but do not directly receive information from the hypothalamic attack area. Some other brain areas may receive converging hypothalamic and septal afferents. The further study on the specific brain areas where serotonergic agents affect hypothalamically elicited aggressive behaviour, may be important for our understanding of uncontrollable aggressive behaviour in humans.

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PART 4 GENERAL DISCUSSION AND SUMMARY

4.1 GENERAL DISCUSSION AND SUMMARY.

The role of the hypothalamus in the regulation of behaviour has received much attention during the last century. By using electrical stimulation and lesioning of different parts of the hypothalamus it has become clear that different types of behaviour are regulated by that part of the brain. In general, most types of behaviour that are directly involved in the survival of both the individual and the species are regulated by the hypothalamus in one way or another. Different types of behaviour are regulated by different parts of the hypothalamus. In the present thesis, behavioural and anatomical aspects of the hypothalamic contribution to the neuronal organization of behaviour have been studied in a multidisciplinary approach. By using 1) local chemical stimulation, 2) behavioural analysis of the elicited behaviour, and 3) a study of the efferent projections, that may be related to specific types of behaviour, especially grooming behaviour and attack behaviour, some general conclusions can be drawn concerning the neuronal substrate of hypothalamically elicited behaviour.

1. Hypothalamic cell bodies are involved in hypothalamically elicited behaviour.

In order to find whether neuronal cell bodies were involved in electrical stimulation-induced grooming behaviour, as it was reported by Lammers et al. [13], a study was performed on the behavioural changes after neuronal cell body stimulation by local injection of non-toxic doses of kainic acid in the rostral parts of the hypothalamus (chapter 2.3). The results have shown an increase in grooming behaviour. In chapter 2.4, the results of experiments in which grooming behaviour was elicited by local injection of NMDA into the hypothalamic "grooming area" have been presented. On the basis of the results presented in chapter 2.3 and 2.4, it is likely, that excitatory amino acids acting on hypothalamic neurons play a role in the elicitation of grooming behaviour.

The fact that some grooming can also be elicited by saline injections (chapter 2.5) and even by insertion of an injection cannula (Van Erp et al., submitted) in specific parts of the "grooming area" may be the result of mechanical manipulation of the brain tissue in the grooming area. This suggests, that intrinsic factors are capable of eliciting grooming behaviour. Whether this response is based on manipulation of afferent axonal endings in this area, on the release of neuroactive substances by dendritic release, or on destruction of neurons whereby neuroactive substances are released, is not clear at the present. In one way or another, hypothalamic neurons play a role in the response.

In this thesis it is also shown that digging, gnawing, drinking and attack, which can be induced by electrical stimulation of other parts of the hypothalamus [11,12,13,14], can also be induced by local infusion of GABA antagonists (chapter 3.4).

2. The structure of hypothalamically elicited behaviour is stimulation- dependent.

In the analysis of grooming behaviour as it has been elicited by NMDA and saline, specific differences in the structure of grooming behaviour have been found (chapter 2.5). These differences were observed in the frequency of grooming elements and in the occurrence of genital grooming and yawning after injection of NMDA. Scratching was not changed in both groups, while it appeared to be reduced after electrical stimulation [21] and kainic acid injections (pers. obs.). Taken together, this indicates, that within the hypothalamic grooming area, different elements of grooming can be selectively elicited or modified (chapter 2.5).

Since different types of stimulation of the hypothalamic "grooming area" (HGA) can elicit structurally different grooming responses, it seems likely that stimulation of the hypothalamic grooming area does not elicit a fixed motor response. Activating the HGA results in changes in the neuronal activity in parts of the central and autonomic nervous system, as well as in the endocrine status of the animal. Apparently, different forms of activation of the HGA have a different effect on the neuronal substrate in the HGA, which becomes manifest in differences in the structure of the displayed grooming behaviour. Therefore, the HGA should be considered as an integrative centre that influences motor output in addition to endocrine and autonomic effects.

3. Behaviourally defined hypothalamic areas are not cytoarchitectonically defined areas.

The hypothalamic areas where different types of behaviour can be elicited do not coincide with cytoarchitectonic areas. The area where grooming can be elicited by chemical stimulation with low doses of kainic acid has been determined as comprising parts of the paraventricular nucleus and of the adjacent dorsal hypothalamic area (chapter 2.3). However, the HGA is not delineated by the boundaries of these cytoarchitectonically defined areas. The areas, in which digging, gnawing, drinking and attack could be elicited are located in different parts of the ventral part of the hypothalamus. The areas where different types of behaviour were elicited by chemical stimulation are similar to the areas where electrical stimulation elicits the same response [11,12,13,14]. Attack was elicited by chemical stimulation of an area lateral to the ventromedial hypothalamic nucleus. The centre of the area where chemical stimulation resulted in drinking, was found ventral to the fornix and dorsolateral to the area where attack was elicited, but these areas were overlapping. Gnawing could be elicited by stimulation of parts of the lateral hypothalamic area, while digging was elicited by stimulation of an area medial to the "gnawing area". Each of those types of behaviour therefore appear to have their own location within the hypothalamus.

The areas where different behaviours can be elicited, show considerable overlap. The overlap in behaviourally defined areas manifested itself by the display of two types of behaviour, either intermittently or successively within the observation period after injection. The overlapping pattern in behaviourally defined areas indicates that no clearly delineated hypothalamic centres for different types of behaviour exist. The diffusely organized network of behaviourally relevant areas within the hypothalamus may be indicative for the complexity of the neuronal network underlying the regulation of specific types of behaviour that occur upon specific internal or external signals. Experimental

manipulation of hypothalamic areas, although very sophisticated and refined, will always affect a combination of different behavioural and autonomic systems. This stresses the importance of detailed mapping of injection- or stimulation sites, together with a detailed analysis of the behavioural change elicited by these manipulations.

4. Hypothalamic efferents can be characterized as thin, highly varicose fibres that form "open pathways".

The use of the neuronal tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) has enabled us to study some morphological characteristics of hypothalamic efferent fibres. From the injection site, hypothalamic efferent fibres spread into various directions. They are usually thin with a large number of "en passant" varicosities. The number of varicosities differ when different areas of passage are compared. The occurrence of these varicosities suggests that information transfer "en passant" is possible in many parts of the brain. This indicates that the brain circuitries involved in the regulation of behaviour, can not be described in terms of simple wiring diagrams. The hypothalamus is part of a continuum of brain areas that are interconnected by a complex network of thin, unmyelinated and varicose fibres. This continuum, referred to as the "core of the neuraxis" [19], can be regarded as the part of the brain involved in the integration of behavioural, endocrine and autonomic responses as a result of different internal and external signals. A large number of neuropeptides present in this brain system, next to the occurrence of monoamines, may play a role in motivational changes over periods of time, leading to overt behavioural changes. Through the large number of "en passant" information transfer points, the activity of many parts of the brain can be adapted to changes in motivational states at the same time.

5. Hypothalamic efferents form diffuse fibre streams.

The efferent fibres of the hypothalamus are not organized in discrete bundles, but rather tend to spread throughout a large part of the brain in diffuse fibre "streams" (chapters 2.6 and 3.5). The fibre streams have been distinghuished on the basis of their location in the brain and the innervation of different brain areas. However, fibres crossing from one fibre stream to another have been seen quite frequently. In general, three ascending fibre "streams" are distinghuished: 1) a dorsal ascending stream, located in the dorsomedial corner of the hypothalamus and entering the bed nucleus of the stria terminalis 2) a ventromedial ascending fibre stream entering the medial preoptic area and 3) a lateral ascending fibre stream, directed towards the lateral septal nucleus. Two thalamic fibre "streams" have been distinghuished: a dorsal and a ventral thalamic fibre stream. Three descending fibre streams have been distinghuished: 1) a dorsal fibre stream to the posterior hypothalamic area and mesencephalic central gray, 2) a ventral fibre stream, caudally continued in the ventral tegmental area and ventromedial parts of the brainstem, and 3) a lateral descending fibre stream, which is caudally continued in the ventrolateral aspects of the central tegmental field and of the brainstem. The dorsal descending fibre stream contributes to the fasciculus longitudinalis of Schütz [20]. The ventral and lateral descending fibre streams may be regarded as caudal continuations of the medial forebrain bundle [18].

The number of fibres from different hypothalamic areas that are found in each fibre stream, differ: ascending fibres from the hypothalamic "grooming area" (HGA) tend to cluster more in the ventromedial than in the lateral ascending fibre stream, while fibres from the hypothalamic "attack area" (HAA) tend to cluster more in the lateral than in the ventromedial ascending fibre stream. In addition, descending fibres from the hypothalamic HGA are mainly found in the dorsal descending fibre stream, running through the central gray, and in the ventral descending fibre stream, running through the ventral tegmental area. Fibres from the hypothalamic HAA are mainly found in the dorsal descending fibre stream and in the lateral descending fibre stream, running through the ventral part of the central tegmental field.

The ascending hypothalamic fibre streams may transfer "behaviourally relevant" information from the hypothalamus to other parts of the limbic system for further evaluation and for comparison with cognitive information from other parts of the brain. In such a way the hypothalamus modulates not only the activity of motor systems that are positioned caudal to the hypothalamus, but also the activity of higher limbic areas.

The descending fibre streams are involved in the actual visceromotor, endocrine and skeletomotor responses that are necessary for the proper performance of the behaviour. Interesting in this respect are the differences in efferents from the HGA and HAA. Descending fibre streams from the HAA appear to end at the level of the pontine central gray, while fibres from the HGA are found as caudal as the nucleus of the solitary tract and descend into the spinal cord. Grooming behaviour has been elicited as caudal as the nucleus of the solitary tract [9,10], while aggressive behaviour could only be elicited by stimulation of brain areas rostral to the pontine central gray [3,4,12,14,16]. It seems likely, that the HGA projects more directly on motor centres that are involved in the performance of grooming behaviour, while the HAA projects to other integrative centres of which the central gray appears to be of importance, rather than directly to skeletomotor output centres.

It remains to be elucidated in which way each fibre stream contributes to the behavioural response that is elicited by hypothalamic stimulation. It is likely that the large number of ascending fibres to the septum and bed nucleus of the stria terminalis are somehow involved in limbic and cognitive aspects of the hypothalamically elicited behaviour. The hypothalamus also strongly projects to the brain stem, through different fibre streams. It is conceivable that each fibre stream has its own function in the integrated response. Especially the efferent fibre streams through the central gray and through the ventral tegmental area may be interesting for further study on the exact role of these streams in the execution of hypothalamically elicited grooming behaviour. It has been reported, that opioid injections in the ventral tegmental area and central gray have opposite effects on feeding behaviour elicited by lateral hypothalamic stimulation [8]. This suggests that different efferent fibre streams may subserve different, even opposite functions.

6. Each behaviourally defined hypothalamic area has its own combination of efferent projections.

A large number of PHA-L injections have been made in different parts of the hypothalamus. The efferents of different parts of the hypothalamic "grooming area" (HGA) have been compared with each other as well as with efferents of hypothalamic areas outside the HGA (chapter 2.6). The same approach was followed for the efferents of the

hypothalamic "attack area" (HAA, chapter 3.5). A large number of projection areas receive afferents from many hypothalamic sites, including the HGA and HAA. The presence of these common efferent projection sites indicate that different behavioural responses use the same functional systems, like visceromotor, endocrine, sensorimotor, skeletomotor, and limbic systems. In what way the activity of these brain areas is changed may depend on the specific location of the hypothalamic site that is stimulated and adapted to the type of behaviour to be displayed.

In chapters 2.6 and 3.5, the efferent projection areas of the HGA) and the HAA have been described. The study on the efferent connections of behaviourally defined hypothalamic areas has indicated that a number of projections arise preferentially from the HGA or from the HAA (chapter 2.6 and 3.5). The similarities and differences between the efferents of these hypothalamic areas is of interest for further study. A simplified schematic comparison of efferents that are preferentially originating in the HGA with those originating in the HAA, is presented in figure 1.

The hypothalamic projections to the septum are clearly topographically organized, in such a way that efferents from different hypothalamic areas innervate different parts of the septum: fibres from the HGA terminate in the ventral part of the lateral septal nucleus, while fibres from the HAA terminate in the dorsolateral aspect of the intermediate part of the lateral septal nucleus, forming pericellular baskets. Within the preoptic area, fibres from the HGA are found in the median preoptic nucleus and in close proximity to the organum vasculosum of the lamina terminalis, within the anteroventral part of the periventricular nucleus. Fibres from the HAA are not found in this area. Within the hypothalamus, fibres from the HGA tend to avoid the ventromedial hypothalamic nucleus, while fibres from the HAA specifically innervate this nucleus. The arcuate nucleus and median eminence receive large numbers of fibres from the HGA, while only sparse numbers of fibres from the HAA are found in these areas.

The HAA projects extensively to the mediodorsal thalamic nucleus and parataenial nucleus, while the HGA projects only sparsely to these nuclei.

Different parts of the hypothalamus project to different parts of the central gray. Fibres from the HGA preferentially innervate the ventral aspects of the lateral part of the central gray, while fibres from the HAA innervate the dorsal aspects of the lateral part of the central gray. Both hypothalamic areas project to the dorsal part of the central gray. Stimulation of different parts of the central gray results in either cardiovascular pressor or depressor responses. Interestingly, fibres from the HGA innervate preferentially the "depressor area", while fibres from the HAA preferentially innervate the "pressor area" in the CG.

The ventral tegmental area is strongly innervated by fibres from the HGA, but not by fibres from the HAA. Within the brain stem, a larger projection from the HGA than from the HAA to the locus coeruleus, raphe magnus, nucleus of the solitary tract and dorsal motor nucleus of the vagal nerve is found.

It is clear that behaviourally defined hypothalamic areas each have a specific set of connections with other brain areas. This specific set of efferent connections is important for the proper performance of the behavioural response.

7. The putative "grooming-" and "attack-" relevant circuitries

Grooming behaviour and attack behaviour are types of behaviour that are displayed under very different circumstances. Both types of behaviour require almost opposite autonomic settings. Both hypothalamic "grooming area" HAA and "attack area" (HGA) have their own set of efferent connections to other brain areas. Not much is known of the role of different parts of the brain, that receive afferent projections from these hypothalamic areas in the regulation of these different types of behaviour, and additional behavioural studies are needed to assess the specific role of these brain areas. However, on the basis of the results of the anatomical studies presented in chapters 2.6 and 3.5, together with data from the literature, some remarks can be made on the anatomic connections involved in the regulation of both grooming and attack behaviour.

The HGA is found to project largely to the septum, preferentially to the ventral part of the lateral septal nucleus, to the bed nucleus of the stria terminalis, to the central gray and to the ventral tegmental area. The ascending connections are probably involved in "motivational" aspects of grooming behaviour, although not much is known about the specific effects of these ascending connections in the regulation of grooming.

The HGA also strongly projects to the anteroventral part of the periventricular nucleus, close to the organum vasculosum of the lamina terminalis (OVLT). The OVLT is capable of transferring information from the blood stream to the central nervous system. The connection of the HGA to this area may be involved in the regulation of this information process by controlling the activity of the OVLT.

The descending projections of the HGA are involved in the visceromotoric and skeletomotoric aspects of grooming behaviour. Grooming behaviour is regarded to be involved in dearousal [5]. It is therefore understandable that a number of areas that receive afferent projections from the HGA, play a major role in the autonomic nervous system, e.g. the nucleus of the solitary tract and the lateral parabrachial nucleus. The specific part of the central gray that receives afferent projections from the HGA, is involved in depressor responses [5]. The projection of the HGA to the locus coeruleus (LC) may be involved in dearousal as well. It has been reported that grooming behaviour concurs with a decrease in locus coeruleus neuron activity [2]. The activity of LC neurons has been found to be a marker of the state of arousal of the animal. If the connection of the HGA to the LC is involved in the reduction of LC neuron activity by grooming behaviour, it may form an important connection in the dearousal properties of grooming behaviour.

The HAA projects to a specific part of the lateral septal nucleus, viz. the dorsolateral portion of the intermediate part of the lateral septal nucleus (chapter 3.5). Lesioning of this septal area results in uncontrolled "rage behaviour" [1]. The inhibitory control of the septal area on hypothalamic activity has been reported before. Less attention has been paid to the reciprocal connection from hypothalamus to the septum in the regulation of aggressive behaviour. Within the septum, memory as well as other information from the hippocampus can be added to motivational information from the hypothalamus. Efferents from the septal area, containing this integrated information, project back to the HAA and can exert an inhibitory control over hypothalamically elicited attack.

The preferential connection of the HAA to the mediodorsal thalamic nucleus (MD) has been indicated in chapter 3.5. The mediodorsal thalamic nucleus plays a facilitatory role in

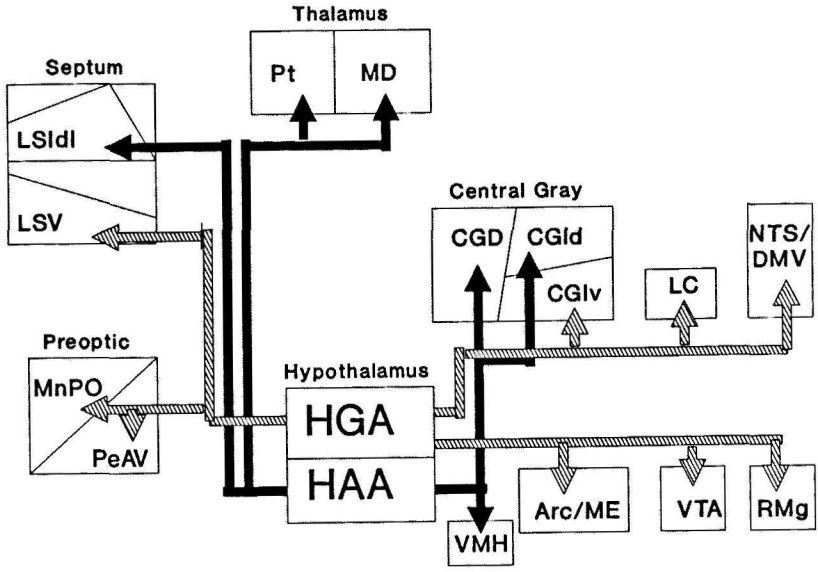

Figure 1. Schematic presentation of efferent connections that originate preferentially in the hypothalamic "grooming area" (HGA) and hypothalamic "attack area" (HAA). Abbreviations: Arc: arcuate nucleus; CGD: dorsal part of the central gray; CGId: dorsal portion of the lateral part of the central gray; CGIv: ventral portion of the lateral part of the central gray; DMV: dorsal motor nucleus of the vagal nerve; LC: locus coeruleus; LSIdl: dosolateral portion of the intermediate part of the lateral septal nucleus; LSV: ventral part of the lateral septal nucleus; MD: mediodorsal thalamic nucleus; ME: median eminence; MnPO: median preoptic nucleus; NTS: nucleus of the solitary tract; PeAV: anteroventral part of the periventricular nucleus; Pt: parataenial thalamic nucleus; RMg: raphe magnus; VMH: ventromedial hypothalamic nucleus; VTA: ventral tegmental area.

the regulation of attack behaviour [3]. The MD projects to the prefrontal cortex, a brain area, that has been suggested to exert an inhibitory control over hypothalamically elicited attack behaviour [6]. This anatomical loop from hypothalamus via MD to prefrontal cortex and back to the HAA may be important in cognitive aspects of aggressive behaviour. The involvement of the MD on hypothalamically elicited attack behaviour through the connections of the MD with the central gray, along the pathway indicated in the chapter 3.5 as the dorsal thalamic fibre stream, may also be of importance.

Only a few connections from the hypothalamic attack area to the amygdala have been found. Yet the medial part of the amydala is known to be involved in the regulation of agonistic behaviour and has strong efferent projections to the hypothalamic attack area [15].

The amygdala has therefore an important role in the regulation of the activity of the hypothalamic area involved in attack and is not directly controlled by the hypothalamic attack area.

The HAA preferentially projects to the part of the central gray, that is involved in pressor responses [5]. Stimulation of this part of the CG elicits attack behaviour as well [16]. Therefore, the central gray can be regarded as a brain area involved in both visceromotor and skeletomotor aspects of hypothalamically controlled attack behaviour. However, lesioning of the CG reduces only slightly hypothalamically elicited attack behaviour [17]. Therefore, other decending pathways, such as the lateral descending fibre stream, may also be involved.

Final remarks

There are still many questions to be answered before a full description can be presented of the exact role of the hypothalamus in the regulation of behaviour and the pathways through which this role is executed. The results that have been presented in the present thesis, show that only very refined neuroanatomical studies in combination with a detailed analysis of the behavioural responses will make it possible to unravel the neuroanatomical pathways that are involved in these circuitries.

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SAMENVATTING.

De rol van de hypothalamus heeft de laatste eeuw veel aandacht gekregen. Door het gebruik van electrische stimulatie en lesie technieken van verschillende delen van de hypothalamus is het duidelijk geworden dat aan aantal verschillende gedragingen gereguleerd worden door dat deel van het brein. In het algemeen worden alle vormen van gedrag die betrokken zijn bij de overleving van het individu en de soort op één of andere manier door de hypothalamus gestuurd. Verschillende gedragingen worden gereguleerd door verschillende delen van de hypothalamus. In dit proefschrift zijn anatomische en gedragsmatige aspecten van het aandeel dat de hypothalamus heeft in de regulatie van gedrag bestudeerd in een multidisciplinaire benadering. Door 1) het gebruik van chemische stimulatie van verschillende delen van de hypothalamus, 2) nauwkeurige analyse van de opgewekte respons, en 3) het onderzoeken van de efferente verbindingen die betrokken zijn bij verschillende vormen van gedrag, nl. poetsgedrag en aggressief gedrag, is een aantal algemene conclusies getrokken ten aanzien van het neuronaal substraat van hypothalamisch geïnduceerd gedrag.

1. Cellichamen in de hypothalamus zijn betrokken bij hypothalamisch geïnduceerd gedrag.

Om na te gaan of neuronale cellichamen betrokken zijn bij electrische stimulatie geïnduceerd poetsgedrag, zoals door Lammers et al. gerapporteerd is, werd een studie gedaan naar de gedragsveranderingen na neuronale cellichaam-stimulatie door het lokaal injecteren van niet- toxische doses kaïnezuur in het rostrale deel van de hypothalamus (hoofdstuk 2.3). De resultaten tonen aan dat deze injecties tot een verhoging van het poetsgedrag leiden. In hoofdstuk 2.4 zijn de resultaten weergegeven van de experimenten waarbij poetsgedrag opgewekt is door lokale injectie van NMDA. Gezien de resultaten van de hoofdstukken 2.3 en 2.4 is het aannemelijk dat excitatoire aminozuren die aangrijpen op hypothalamische neuronen, een rol spelen bij het opwekken van poetsgedrag.

De bevinding dat het injecteren van fysiologisch zout (hoofdstuk 2.5) en zelfs het insteken van een injectiecanule (Van Erp et al.) in een gedeelte van het hypothalamisch "poetsgebied" poetsgedrag versterkt, zou het resultaat kunnen zijn van het mechanisch stimuleren van het hersenweefsel in het poetsgebied. Dit zou kunnen betekenen dat locale factoren in staat zijn om plaatselijk neuronen te stimuleren. Of deze stimulatie het gevolg is van 1) manipulatie van afferente axoneindigingen in dit gebied, 2) de afgifte van neuroactieve stoffen via dendrieten of 3) het vernielen van cellichamen waardoor actieve stoffen in de vrije ruimte vrijkomen, is niet bekend. In ieder geval zijn ook hier cellichamen bij betrokken.

In dit proefschrift is ook aangetoond dat graven, knagen, drinken en agressief gedrag, gedragingen die alle opgewekt kunnen worden door electrische stimulatie van andere delen

van de hypothalamus, ook opgewekt kunnen worden door het lokaal infunderen van GABA antagonisten (hoofdstuk 3.4).

2. De structuur van hypothalamisch geïnduceerd gedrag is stimulatie-afhankelijk.

In de analyse van poetsgedrag dat is opgewekt door NMDA- en fysiologisch zoutinjecties, zijn verschillen in de structuur van dit gedrag gevonden (hoofdstuk 2.5). Deze verschillen werden gevonden in de frequentie van de poetselementen en in het optreden van genitaalpoetsen en gapen na injectie met NMDA. Krabben was niet veranderd, terwijl andere vormen van stimulatie aangaven dat dit element juist vermindert door electrische stimulatie (Van Erp et al. 1991) en het injecteren van kaïnezuur (eigen waarneming). Deze verschillen in poetsrespons wijzen erop dat binnen de hypothalamus verschillende elementen van poetsgedrag selectief kunnen worden opgewekt of veranderd.

Aangezien verschillende manieren van stimulatie van het hypothalamische "poetsgebied" (HGA) structureel verschillende vormen van poetsen op kunnen wekken, is het waarschijnlijk dat stimulatie van het HGA niet een vastgelegde motorrespons opwekt. Het activeren van het HGA resulteert in veranderingen in neuronale activiteit in verschillende delen van het centrale en autonome zenuwstelsel, alsmede in de endocriene staat van het dier. Klaarblijkelijk hebben verschillende vormen van activatie verschillende effecten op het neuronaal substraat in het HGA, hetgeen tot uitdrukking komt in verschillen in de structuur van het getoonde poetsgedrag. Daarom moet het HGA beschouwd worden als een integratiecentrum dat motorische centra beïnvloed naast endocriene en autonome effecten.

3. Gedragsmatig gedefiniëerde gebieden van de hypothalamus zijn geen cytoarchitectonisch gedefiniëerde gebieden.

De verschillende gebieden waar verschillende vormen van gedrag opgewekt kunnen worden, komen niet overeen met cytoarchitectonische gebieden. In hoofdstuk 2.3 is aangegeven hoe met behulp van locale injecties met kaïnezuur, het hypothalamische "poetsgebied" (HGA) omschreven kan worden als een gebied dat bestaat uit delen van de nucleus paraventricularis en het aangrenzende dorsale hypothalamusgebied. Maar het HGA wordt niet omgrensd door de omtrekken van deze cytoarchitectonische gebieden. De gebieden waar graven, knagen, drinken en agressief gedrag opgewekt konden worden, liggen in verschillende delen van het ventrale deel van de hypothalamus. De gebieden, waar deze responsen opgewekt konden worden met chemische stimulatie zijn hetzelfde als de gebieden waar met electrische stimulatie dezelfde responsen opgewekt konden worden. Agressief gedrag kon opgewekt worden door chemische stimulatie van het gebied dat lateraal ligt van de nucleus ventromedialis hypothalami. Het centrum van het gebied waar drinken opgewekt kon worden, ligt ventraal van de fornix en dorsolateraal van het gebied waar agressie opgewekt kon worden, maar deze twee gebieden zijn wel overlappend. Knagen kan opgewekt worden door stimulatie van delen van de laterale hypothalamus, terwijl graven opgewekt kon worden door stimulatie van een gebied, dat mediaal lag ten opzichte van het hypothalamische "knaaggebied".

De verschillende "gedragsgebieden" vertonen een grote overlap. Deze overlap

manifesteert zich door het opwekken van twee verschillende vormen van gedrag na één enkele injectie in dezelfde periode van registratie alsmede door het opwekken van twee vormen van gedrag successievelijk binnen de observatieperiode. Deze overlappende patronen geven aan dat er geen duidelijke afgrensbare centra in de hypothalamus bestaan voor verschillende vormen van gedrag. Het diffuus georganiseerde netwerk van gedragsgebieden in de hypothalamus geeft de complexiteit aan van de neuronale netwerken die betrokken zijn bij de regulatie van specifieke vormen van gedrag, die vertoond worden na specifieke interne en/ of externe stimuli. Het experimenteel manipuleren van de hypothalamus zal, hoe nauwkeurig en verfijnd ook, altijd verschillende gedrags- en autonome centra beïnvloeden. Het is daarom van groot belang injectie- of stimulatieplaatsen nauwkeurig in een atlas weer te geven, naast het nauwkeurig analyseren van de gedragsveranderingen als gevolg van die manipulaties.

4. Hypothalamische efferenten kunnen gekenmerkt worden als dunne, hoog variceuze vezels, die tesamen een "open baansysteem" vormen.

Door het gebruik van de neuronale tracer *Phaseolus vulgaris* leucoagglutinine hebben we een studie kunnen maken van de kenmerken van hypothalamische efferenten. Van de injectieplaats af verspreiden hypothalamische vezels zich in verschillende richtingen. Deze vezels zijn gewoonlijk dun en bevatten een groot aantal "en passant" varicositeiten. Het aantal varicositeiten verschilt bij vergelijking tussen verschillende gebieden die door die vezels doorkruist worden. Het voorkomen van deze varicositeiten geeft aan, dat informatie-overdracht mogelijk is in een groot aantal gebieden dat doorkruist wordt: hersencircuits die betrokken zijn bij de regulatie van gedrag moeten klaarblijkelijk niet beschouwd worden als eenvoudige bedradingsschema's. De hypothalamus is een deel van een continuum van hersengebieden die onderling verbonden zijn door een complex netwerk van dunne, ongemyeliniseerde en variceuze vezels. Dit continuum, dat aangeduid is als de "kern van de neurale as" (Nieuwenhuys, 1985), kan beschouwd worden als dat deel van het brein dat betrokken is bij de integratie van gedragsmatige, endocriene en autonome responsen op verschillende interne en externe signalen. Vele van de neuropeptiden die in dit hersensysteem voorkomen, alsmede monoamines, zouden een rol kunnen spelen in de motivationele veranderingen over een langere tijdspanne, met gedragsveranderingen als gevolg. Door de grote hoeveelheid "en passant" informatie overdrachtspunten, in de vorm van varicositeiten, kan de activiteit van veel verschillende hersengebieden mogelijk tegelijkertijd aangepast worden aan veranderingen in de motivationele status van het dier.

5. Hypothalamische efferenten vormen diffuse vezelstromen.

De uitgaande verbindingen van de hypothalamus vormen geen concrete vezelbundels, maar waaieren uit over een groot gedeelte van het brein in diffuse "vezelstromen" (hoofdstukken 2.6, 3.5). Deze vezelstromen zijn aangeduid op basis van hun locatie in het brein en de innervatie van veschillende hersengebieden. Deze aanduidingen zijn niet strikt, aangezien vrij frequent vezels werden aangetroffen die van de ene naar de andere vezelstroom oversteken. Over het algemeen zijn er drie opstijgende vezelstromen benoemd: 1) een dorsale, gelegen in de dorsomediale hoek van de hypothalamus, die naar de

bed nucleus van de stria terminalis loopt, 2) een ventromediale, die in het mediale preoptische gebied gelegen is, en 3) een laterale, die naar het septum toe gaat. Twee vezelstromen lopen naar de thalamus een dorsale en een ventrale thalamische vezelstroom Drie afdalende vezelstromen zijn onderscheiden: 1) een dorsale afdalende vezelstroom naar het posterior hypothalamusgebied en mesencephale centrale grijs, 2) een ventrale stroom, die caudaal voortgezet wordt in de area tegmentalis ventralis en ventromediale delen van de hersenstam, en 3) een laterale afdalende stroom, die caudaal voortgezet wordt in de ventrolaterale delen van het centrale tegmentale veld en van de hersenstam. De dorsale afdalende stroom is onderdeel van de fasciculus longitudinalis van Schutz, de twee andere kunnen gezien worden als caudale voortzettingen van de mediale voorhersenbundel.

Het aantal vezels in de opstijgende en afdalende vezelstromen vanuit verschillende delen van de hypothalamus is niet hetzelfde opstijgende vezels vanuit het hypothalamische "poetsgebied" (HGA) lijken zich meer te verzamelen in de ventromediale dan in de laterale opstijgende vezelstroom, terwijl vezels uit het hypothalamische "agressiegebied" (HAA) meer lijken te clusteren in de laterale opstijgende stroom dan in de ventromediale opstijgende vezelstroom Afdalende vezels van het HGA bevinden zich voornamelijk in de dorsale afdalende stroom naar het centrale grijs en in de ventrale afdalende stroom door de area tegmentalis ventralis Afdalende vezels vanuit het HAA bevinden zich voornamelijk in de dorsale en laterale afdalende vezelstromen die door het centrale grijs en ventrale delen van het centrale tegmentale veld verlopen.

De opstijgende vezelstromen zouden gedragsmatig relevante informatie vanuit de hypothalamus door kunnen geven aan andere delen van het limbische systeem waar deze informatie vergeleken en gewogen kan worden met informatie uit andere hersendelen, waaronder cognitieve en geheugeninformatie Op deze manier is de hypothalamus niet alleen in staat om de activiteit van motorische centra in de hersenstam te beinvloeden, maar ook de activiteit van hogere, limbische centra

De afdalende vezelstromen zijn betrokken bij de daadwerkelijke visceromotorische, endocriene en skeletomotorische responsen die nodig zijn voor het goed uitvoeren van de gedragsrespons. In dit opzicht zijn de verschillen tussen afdalende vezels van het HGA en het HAA interessant. Afdalende vezelstromen vanuit het HAA lijken te eindigen ter hoogte van het centrale grijs van de pons, terwijl vezels vanuit het HGA doorlopen tot aan de nucleus van de tractus solitarius en verder het ruggemerg in Poetsgedrag is opgewekt door stimulatie van ver caudaal gelegen hersengebieden, zoals genoemde nucleus van de tractus solitarius, terwijl agressie alleen opgewekt wordt door stimulatie van hersendelen die rostraal van het centrale grijs van de pons gelegen zijn Het lijkt er op dat het HGA directer op motorische centra die betrokken zijn bij het uitvoeren van poetsgedrag projecteert, terwijl het HAA meer projecteert naar andere integratieve centra, met name het centrale grijs in plaats van direct naar skeletomotorische centra

Het is vooralsnog onbekend op welke manier elke vezelstroom bijdraagt aan de gedragsrespons die opgewekt is door stimulatie van de hypothalamus. Dat het grote aantal opstijgende vezels naar de bed nucleus van de stria terminalis en het septum iets te maken heeft met limbische en cognitieve aspecten van hypothalamisch geinduceerd gedrag, is erg waarschijnlijk De hypothalamus projecteert ook sterk naar de hersenstam, via verschillende vezelstromen Het is mogelijk dat elke vezelstroom een specifieke functie heeft bij de uitvoering van een geintegreerde response De vezelstromen door het centrale grijs en door de area tegmentalis ventralis zouden hierbij interessant kunnen zijn voor verdere studie aan

de exacte rol van deze twee stromen in de uitvoer van hypothalamisch opgewekt poetsgedrag. Het is bekend dat injecties met opioïden in het centrale grijs en de area tegmentalis ventralis tegenovergestelde effecten hebben op eetgedrag dat opgewekt is door electrische stimulatie van de delen van de laterale hypothalamus. Dit zou kunnen betekenen dat verschillende vezelstromen verschillende, of zelfs tegenovergestelde functies hebben.

6. Elke gedragsmatig gedefiniëerd hypothalamusgebied heeft zijn eigen combinatie van efferente projecties.

Er is een groot aantal PHA-L injecties gemaakt in verschillende delen van de hypothalamus. De efferente verbindingen van verschillende delen van het hypothalamische "poetsgebied" (HGA) zijn vergeleken met elkaar en met de verbindingen van delen van de hypothalamus, die buiten het HGA liggen (hoofdstuk 2.6). Een dergelijke benadering is ook gebruikt bij de verbindingen van het hypothalamische "agressiegebied" (HAA). Veel projectiegebieden krijgen informatie uit verschillende delen van de hypothalamus, waaronder het HGA en het HAA. Het bestaan van deze overeenkomstige projectiegebieden zou een aanwijzing kunnen zijn dat verschillende gedragsresponsen gebruik maken van dezelfde functionele systemen, zoals visceromotorische, endocriene, sensorimotorische, skeletomotorische en limbische systemen. Op welke manier de activiteit van deze gebieden veranderd wordt kan afhankelijk zijn van de specifieke locatie van een gebied in de hypothalamus dat geactiveerd wordt, en aangepast worden aan het soort gedrag dat uitgevoerd gaat worden.

In de hoofdstukken 2.6 en 3.5 zijn de efferenten van het HGA en het HAA beschreven. In die hoofdstukken blijkt dat verschillende projecties vanuit de hypothalamus naar andere delen van het brein bij voorkeur uit het HGA of uit het HAA komen. De verschillen en overeenkomsten tussen de efferenten van deze twee hypothalamusgebieden is interessant voor verdere bestudering. Een vereenvoudigd overzicht van de verbindingen, die bij voorkeur uit het HGA komen, tesamen met de verbindingen, die bij voorkeur uit het HAA komen, zijn weergegeven in figuur 1 van de General discussion (Deel 4 van dit proefschrift).

De projecties van de hypothalamus naar het septum zijn duidelijk ruimtelijk georganiseerd: vezels vanuit het HGA eindigen voornamelijk in het ventrale deel van het laterale septum, terwijl vezels uit het HAA voornamelijk eindigen in de dorsolaterale hoek van het intermediaire deel van het laterale septum, waar ze pericellulaire korfjes vormen. Vezels uit het HGA eindigen in het preoptische gebied in de mediane preoptische kern en vlak bij het organum vasculosum van de lamina terminalis, binnen het anteroventrale deel van de periventriculaire kern. Vezels uit het HAA zijn in deze gebieden niet aangetroffen. Binnen de hypothalamus lijken de vezels uit het HGA de ventromediale kern van de hypothalamus te vermijden, terwijl vezels uit het HAA deze kern sterk innerveren. De nucleus arcuatus hypothalami en de eminentia mediana ontvangen zeer veel vezels uit het HGA, terwijl hier slechts enkele vezels uit het HAA zijn aangetroffen.

Het HAA projecteert sterk naar de mediodorsale kern en de nucleus parataenialis van de thalamus, terwijl het HGA hier slechts enkele vezels naar toe stuurt.

Verschillende delen van de hypothalamus projecteren naar verschillende delen van het centrale grijs. Vezels van het HGA innerveren voornamelijk de ventrale hoek van het laterale deel van het centrale grijs, terwijl vezels uit het HAA naar de dorsale hoek van dit

deel van het centrale grijs projecteren. Beide gebieden projecteren naar het dorsale deel van het centrale grijs. Stimulatie van verschillende delen van het centrale grijs resulteert in ofwel cardiovasculaire pressorreacties ofwel depressorreacties. Vezels van het HGA innerveren vooral het "depressorgebied" en vezels vanuit het HAA innerveren voornamelijk het "pressorgebied" in het centrale grijs.

De area tegmentalis ventralis wordt sterk geïnnerveerd door vezels vanuit het HGA maar niet door vezels vanuit het HAA. In de hersenstam zijn de projecties naar de locus coeruleus, raphe magnus, de nucleus van de tractus solitarius en de dorsale motorkern van de nervus vagus vanuit het HGA vele malen groter dan die uit het HAA.

Het moge duidelijk zijn dat gedragsmatig gedefiniëerde hypothalamusgebieden elk een specifieke set van efferente verbindingen met andere hersengebieden heeft. Deze specifieke set van verbindingen is belangrijk voor het goed uitvoeren van de gedragsresponse.

6. De mogelijke "poets-" en "agressie-" relevante circuits

De omstandigheden waarin poetsgedrag en agressief gedrag vertoond worden, zijn erg verschillend van elkaar. Deze twee vormen van gedrag gaan gepaard met bijna tegengestelde interne staten van het dier. Het hypothalamisch "poetsgebied" (HGA) en "agressiegebied" (HAA) hebben elk hun eigen set van efferente verbindingen naar andere hersengebieden. Er is echter niet erg veel bekend over de rol die deze andere hersengebieden hebben in de regulatie van poetsen en agressie en er zijn nog veel gedragsstudies nodig om de specifieke rol van elk van deze hersengebieden te vinden. Op basis van de resultaten die in de hoofdstukken 2.6 en 3.5 gepresenteerd zijn, en in combinatie met gegevens uit de literatuur, kunnen echter toch wel enkele opmerkingen gemaakt worden over de anatomische verbindingen die betrokken zijn bij de regulatie van poetsen en agressie.

Het HGA projecteert uitbundig naar het septum, voornamelijk naar het ventrale deel van het laterale septum, naar de bed nucleus van de stria terminalis, naar het centrale grijs en naar de area tegmentalis ventralis. De opstijgende verbindingen zijn mogelijk betrokken bij "motivationele" aspecten van poetsgedrag, hoewel er nog erg weinig bekend is over de specifieke effecten van deze opstijgende verbindingen in de regulatie van poetsgedrag.

Het HGA projecteert ook sterk naar het anteroventrale deel van de periventricularie kern, vlakbij het organum vasculosum van de lamina terminalis (OVLT). De OVLT is in staat om informatie van signaalstoffen in het bloed te vergaren en door te geven aan het centrale zenuwstelsel. De verbinding vanuit het HGA naar dit gebied zou te maken kunnen hebben met de regulatie van dit informatieproces door de activiteit van dit zintuigorgaan te controleren.

De afdalende verbindingen vanuit het HGA zijn betrokken bij visceromotorische en skeletomotorische aspecten van poetsgedrag. Poetsgedrag lijkt betrokken zijn bij het proces van "dearousal" (Delius, 1970). Het is daarom begrijpelijk dat hersengebieden die projecties ontvangen uit het HGA, zoals de nucleus van de tractus solitarius en de parabrachaiale kernen, een belangrijke rol spelen in het autonome zenuwstelsel. Het deel van het centrale grijs waar het HGA bij voorkeur naartoe projecteert, is betrokken bij cardiovasculaire "depressorreacties" (bloeddrukdaling). De projectie van het HGA naar de

locus coeruleus zou ook bij "dearousal" betrokken kunnen zijn. De activiteit van locus coeruleus-neuronen gaat omlaag als dieren gaan poetsen en de activiteit van deze neuronen is een aanwijzing voor de staat van "arousal" waarin het dier verkeert Aston-Jones et al., 1981). Als de verbinding vanuit het HGA naar de locus coeruleus betrokken is bij de daling van locus coeruleus neuronen-activiteit, dan zou deze verbinding belangrijk kunnen zijn bij de rol die poetsen speelt in "dearousal".

Het HAA projecteert ook naar specifieke delen van het laterale septum, namelijk naar de dorsolaterale hoek van het intermediaire deel van het laterale septum (hoofdstuk 3.5). Het laederen van delen van het laterale septum resulteert in een vorm van agressief gedrag ("rage behaviour"). De remmende rol van het septum op hypothalamische activiteit is reeds bekend. Er is echter veel minder aandacht besteed aan de reciproke verbinding van de hypothalamus naar het septum in de regulatie van agressief gedrag. In het septum zou geheugeninformatie en andere informatie van de hippocampus toegevoegd kunnen worden aan motivationele informatie uit de hypothalamus. De uitgaande verbindingen van het septum die deze geïntegreerde informatie bevatten, projecteren terug naar het HAA en kunnen een inhiberende invloed hebben op hypothalamisch gestuurd agressief gedrag.

De voorkeursverbinding van het HAA naar de mediodorsale kern van de thalamus (MD) is aangegeven in hoofdstuk 3.5. De MD heeft een faciliterende rol in de regulatie van agressie. Deze kern projecteert naar de prefrontale cortex, een hersengebied dat betrokken is bij agressie door zijn inhiberende invloed op hypothalamisch opgewekt agressief gedrag. Deze anatomische kring van HAA via MD naar prefrontale cortex en terug naar het HAA zou van belang kunnen zijn bij de controle van cognitieve aspecten van agressief gedrag. De verbinding van de MD naar het centrale grijs, langs de weg die de dorsale thalamische vezelstroom volgt, zou ook van belang kunnen zijn.

Vanuit het HAA zijn slechts enkele verbindingen naar de amygdala gevonden, terwijl de mediale amygdala ook betrokken is bij de regulatie van agressief gedrag en sterke projecties uitstuurt naar het HAA. De amygdala lijkt daarom een belangrijke rol te spelen bij de regulatie van de activiteit van de neuronen in het HAA, maar wordt blijkbaar niet direct gecontroleerd vanuit de HAA.

Het HAA projecteert sterk naar dat deel van het centrale grijs dat betrokken is bij cardiovasculaire "pressor-responsen" (bloeddrukstijging). Stimulatie van dat deel van het centrale grijs wekt ook agressief gedrag op (Mos et al., 1982). Het centrale grijs is dus waarschijnlijk betrokken bij zowel visceromotorische als skeletomotorische aspecten van hypothalamisch gecontroleerd agressief gedrag. Het is echter wel zo dat het laederen van het centrale grijs slechts ten dele hypothalamisch opgewekt agressief gedrag remt. Daarom zouden nog andere afdalende verbindingen, zoals de laterale afdalende vezelbaan, een rol kunnen spelen.

Laatste opmerkingen

Er dienen nog veel vragen opgelost te worden voordat een volledige beschrijving gegeven kan worden van de exacte rol van de hypothalamus in de regulatie van gedrag en van de wegen waarlangs deze rol wordt vervuld. De reultaten die in de verschillende hoofdstukken van dit proefschrift zijn gepresenteerd, laten zien dat alleen nauwkeurige

neuroanatomische studies tezamen met een gedetailleerde analyse van de gedragsresponsen de neuroanatomische wegen die betrokken zijn bij de regulatie van gedrag, kan doen ontrafelen.

DANKWOORD

Het is al meerdere malen gezegd, dat een proefschrift niet door één persoon alleen geschreven wordt. Daar zijn meerdere mensen bij betrokken. De manier waarop die mensen hebben meegeholpen, is echter soms zó divers, dat het vreemd is deze mensen op een zelfde pagina afgedrukt te zien.

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Annemoon van Erp is in de jaren van dit promotieonderzoek niet alleen een collega geweest, maar een echte vriendin. Annemoon, voor alle dingen, die we samen gedaan en besproken hebben, bedankt !

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Tom

CURRICULUM VITAE

Tom Roeling is geboren op 4 februari 1963, te Den Hoorn bij Delft. Na de lagere school (Mariaschool) en het gymnasium (St. Stanislascollege Delft) te hebben doorlopen begon hij in 1981 aan zijn studie Biologie. In 1985 behaalde hij het kandidaatsexamen biologie. Tijdens zijn doctoraalstudie heeft hij de volgende onderzoeken verricht: 1] de organisatie van het ventraal paleostriatum van de wilde eend (Prof. Dr. J.L. Dubbeldam), 2] De mogelijke rol van glia bij de vorming van longitudinale patronen in de primitieve cerebellaire schors van de kip (Dr. H.K.P. Feirabend), 3] Stress-geïnduceerde analgesie bij ratten: De mogelijke rol van het opioidsysteem (Dr. M.R. Kruk, Prof. Dr. K.A. Miczek). Tevens heeft hij als student geassisteerd bij het practicum morfologie voor eerstejaars biologen en het practicum humane anatomie en neuroanatomie voor medisch biologen en gezondheidswetenschappers. In 1987 behaalde hij het doctoraal examen biologie en begon hij aan het promotieonderzoek "het neuronaal substraat van hypothalamisch geïnduceerd gedrag" (promotor Prof. Dr. R. Nieuwenhuys, co-referenten Dr. J.G. Veening en Dr. M.R. Kruk) binnen het kader van de BION programmasubsidie "De rol van de hypothalamus in de regulatie van gedrag" (430.901P). Vanaf 1 april 1992 is hij tijdelijk werkzaam bij de vakgroep Anatomie en Embryologie van de Vrije Universiteit te Amsterdam (Prof. Dr. H.J. Groenewegen).